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Drinking Water Criteria Document for Ortho-Dichlorobenzene, Meta-Dichlorobenzene, and Para-Dichlorobenzene (Draft)

(U.S.) Environmental Protection Agency
Washington, DC

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DRAFT CRITERIA DOCUMENT
FOR
ORTHO-DICHLOROBENZENE
META-DICHLOROBENZENE
PARA-DICHLOROBENZENE

June 1985

CRITERIA AND STANDARDS DIVISION
OFFICE OF DRINKING WATER
U.S. ENVIRONMENTAL PROTECTION AGENCY
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16. ABSTRACT

The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on o-, m- and p-dichlorobenzene. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of o-, m- and p-dichlorobenzene
- Toxicokinetics and human exposure to o-, m- and p-dichlorobenzene
- Health Effects of o-, m- and p-dichlorobenzene in humans and animals
- Mechanisms of toxicity of o-, m- and p-dichlorobenzene
- Quantification of toxicological effects of o-, m- and p-dichlorobenzene

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I. SUMMARY

There are three isomers of dichlorobenzene: ortho-(1,2-), meta- (1,3-) and para- (1,4-). The major uses of ortho-dichlorobenzene (o-DCB) are as a process solvent in the manufacture of toluene diisocyanate and as an intermediate in the synthesis of dyestuffs, herbicides and degreasers (Ware and West, 1977). The bulk of para-dichlorobenzene (p-DCB) usage is in direct application as an air deodorant or insecticide which accounts for 90% of its total consumption (Lowenheim and Moran, 1975; Ware and West, 1977). The use of o- and p-DCB as deodorizers in industrial wastewaters and toilet bowl waters would suggest that there is potential for these substances to be found in waters throughout the country. No documented uses for meta-dichlorobenzene (m-DCB) were found in the literature.

Ortho- and para- DCB are produced in considerable quantity; production volumes of o-DCB equalled 22,000 kkg and of p-DCB equalled 34,000 kkg in 1981 (USITC, 1981). Environmental releases of the dichlorobenzenes have been estimated at 30,000 kkg (or 57% of production). Approximately 7,000 kkg of o-DCB are released after solvent use and 22,000 kkg p-DCB are released from moth balls and space deodorants (SAI, 1980). Meta-DCB gets into the environment as a breakdown product of certain pesticides and as a byproduct of the manufacture of other chlorinated benzenes.

All three isomers of dichlorobenzene have been detected in drinking water supplies from both ground and surface waters, in quantities ranging from less than 0.5 ug/l to 9 ug/l (EPA, 1975; EPA, 1978a).

No studies have been reported which determine the percentage of a dose of dichlorobenzene absorbed following oral or inhalation exposure. However, for the purpose of regulation development, based upon the absorption characteristics of benzene and the smaller chlorinated ethanes and ethylenes, it will be assumed that 100% of an oral dose of any of the isomers of dichlorobenzene is absorbed and that 60% of an inhalation dose is absorbed when exposure persists for longer than one to three hours (Astrand, 1975;Dallas, et al., 1983).

After oral administration to rabbits, the DCBs are oxidized principally to phenols. Ortho- and m-DCB also form catechols (Azouz, et al., 1955; Williams, 1959). Although small amounts of the metabolites are excreted as free phenols or catechols, the ovewhelming percentage are eliminated as conjugates of glucuronic or sulfuric acids. Ortho- and meta-DCB form mercapturic acids as well, but p-DCB does not (Williams, 1959). The conjugated dichlorophenols appear to be the principal metabolic products of the DCB isomers in man (Hallowell, 1959; Pagnatto and Walkley, 1965).

The ortho- and para- isomers are lipophilic and can be expected to bioaccumulate to some extent, particularly in tissues with high fat content, during prolonged, continuous exposures. Para-DCB has been detected in human adipose tissue and all three isomers have been detected in blood (Dowty, et al., 1975; Morita, et al., 1975; Morita and Ohi, 1975).

Reports have appeared in the literature describing poisoning incidents resulting from exposure to the dichlorobenzenes.

Girard, et al. (1969) reported four cases of leukemia in patients purportedly exposed to varying quantities and mixtures of dichlorobenzene, although each solution contained the ortho isomer. It is possible that these individuals also were exposed to other agents at the same time.

Hallowell (1959), Gadrat, et al. (1962), Girard, et al. (1969) and Campbell and Davidson (1970) all described cases in which individuals suffered from moderate to severe anemia following high levels of exposure to the DCBs. Several instances of skin lesions (e.g., pigmentation and allergic dermatitis) developing after contact also have been reported (Downing, 1939; Frank and Cohen, 1961; Nalbandian and Pearce, 1965).

In other reported cases, patients complained of vomiting, headaches, irritation of the eyes and upper respiratory tract and profuse rhinitis and periorbital swelling (Dupont, 1938; Cotter, 1953; Campbell and Davidson, 1970). Anorexia, nausea, vomiting, weight loss, yellow atrophy of the liver and blood dyscrasias also were reported for higher exposure concentrations (Petit and Champeix, 1954; Cotter, 1953; Wallgren, 1953; Weller and Crellin, 1953; Hallowell, 1959). Liver damage sometimes was accompanied by prophyria (Hallowell, 1959).

The dichlorobenzenes produce sedation and anesthesia in animals after acute oral or parenteral administration. Relatively high doses are needed to produce acute effects. Acute poisoning is characterized by signs of disturbance of the central nervous system: hyperexcitability, restlessness, muscle spasms or tremors. The most frequent cause of death is respiratory depression. Acute exposure also may result in kidney and/or liver damage. Liver alterations may be manifested as necrosis/degeneration, perhaps coincident with porphyria.

The LD_{50} for o-DCB in rats after oral administration ranged from 500 mg/kg (NIOSH, 1978) to 1,500 mg/kg (Hollingsworth, _et al_., 1958). In the guinea pig, the oral LD_{50} was 2,000 mg/kg (Hollingsworth, _et al_., 1958). The oral LD_{50} for p-DCB ranged from 500 mg/kg (NIOSH, 1978) to 2,500 mg/kg (Hollingsworth, _et al_., 1956) in the rat and was 3,220 mg/kg in the mouse (Varshavskaya, 1968). The lowest published lethal oral dose in guinea pigs was 2,800 mg/kg (Hollingsworth, _et al_., 1956). Irie, _et al_. (1973) reported an LD_{50} of 5,145 mg/kg for a subcutaneous injection of p-DCB in the mouse.

Histological studies following acute and subacute exposure to o-DCB have revealed damage of the liver and kidney. Exposing mice to the similar concentrations caused CNS stimulation for about 20 minutes followed by CNS depression, muscular twitching, slow and irregular respiration, cyanosis near the end of an hour and death within 24 hours. Rats appeared to be slightly more resistant than mice to the toxic effects of o-DCB.

Inhalation of 80 ppm o-DCB by rats for 11-50 hours was irritating to the eyes and nose, produced slight changes in the tubular epithelium of the kidney and resulted in confluent necrosis of the liver (Cameron, _et al_., 1937).

Rabbits, rats, and guinea pigs exposed for 20-30 minutes daily to 100 mg p-DCB/liter of air for 5-9 days showed marked irritation of the eyes and nose, muscle twitching, tremors, CNS depression, nystagmus and rapid but labored breathing with each exposure, but recovered within 30-180 minutes after being removed from the p-DCB-rich atmosphere (Zupko and Edwards, 1949). In rats, at the same dose, CNS depression was observed to be greater than in rabbits. Many of the test animals of all three species developed granulocytopenia. However, in these experiments, the granulocytopenia was reversible. When the animals were removed from exposure to p-DCB, the granulocyte count returned to normal within three to four weeks. The question arises as to whether the effects seen in this experiment were due to the DCB or to contamination by benzene or other substances.

Fourteen-day repeated dose gavage studies in mice (30-4000 mg/kg) and rats (60-1000 mg/kg) were conducted with both o- and p-DCB in the prechronic testing phase of the National Toxicology Program (NTP) bioassay on these two substances (Battelle-Columbus, 1978 a,b,d,e,f,g,h). In addition to early deaths and lack of body weight gain at the higher doses, animals exhibited histopathological changes indicative of hepatic centrolobular necrosis and degeneration, occasionally with cyto- and karyomegaly, as well as lymphoid depletion of the spleen and thymus.

Gavage doses of o-DCB at 250 and 500 mg/kg given to rats and mice over a thirteen-week schedule of 5 days/week resulted in hepatic necrosis as well as porphyria (Battelle-Columbus, 1978c, i). Serum GPT levels were increased in mice exhibiting liver histopathology at the highest dose level. Some mice also exhibited myocardial and skeletal muscle mineralization and lymphoid depletion of the thymus and spleen and necrosis of the spleen. Rats also showed pathological changes in their kidneys, characterized by tubular degeneration.

Hollingsworth, et al. (1958) gave rats a series of 138 doses of o-DCB over a period of 192 days (18.8, 188 or 376 mg/kg/day, five days a week) by gastric intubation. No adverse effects were noted at the lowest dose. With the intermediate dose, a slight increase in the weights of the liver and kidney was noted. At the highest dose, there was a moderate increase in the weight of the spleen and cloudy swelling of the liver.

Hollingsworth, et al. (1958) also assessed the effects of multiple inhalation exposures to o-DCB in rats, guinea pigs, mice, rabbits and monkeys. The animals were exposed to a range of concentrations seven hours a day, five days a week, for six to seven months. No adverse effects were observed in rats, guinea pigs or mice exposed to 49 ppm (0.29 mg/l), or in rats, guinea pigs, rabbits and monkeys exposed to 93 ppm (0.56 mg/l).

Twenty oral doses of 10, 100 or 500 mg/kg p-DCB given five days a week to rats produced marked cloudy swelling and centrilobular necrosis only with the highest dose (Hollingsworth, et al., 1958). No adverse effects were observed at the other doses.

Thirteen-week exposures to p-DCB by gavage resulted in histopathological alterations in the liver similar to those observed with o-DCB, but at somewhat higher doses (675 and 800 mg/kg in the mouse, 300 and 600 mg/kg in the rat)(Battelle-Columbus, 1978a,b,1980 a,b). Hepatic necrosis, degeneration and porphyria were found in both species. The spleen and thymus also exhibited histopathological changes similar to those observed after o-DCB.

In mice and rats, hematopoietic hypoplasia of the bone marrow occurred in survivors at the highest dose (1500 mg/kg/day). Rats at the two highest dose levels (1000 and 1500 mg/kg) also exhibited epithelial necrosis of the nasal turbinates and small intestine as well as villar bridging of the mucosa of the latter tissue. Again, the rats exhibited multifocal degeneration or necrosis of the cortical tubular epithelium of the kidney.

Oral doses of 188 or 376 mg p-DCB/kg given five days a week, for 192 days (138 doses) to rats produced an increase in the weights of the liver and kidneys (Hollingsworth, et al., 1956). At 376 mg/kg, increased splenic weight, slight cirrhosis and focal necrosis of the liver were observed. No adverse effects were seen with the 18.8 mg/kg dose.

Inhalation studies also were carried by Hollingsworth, et al. (1956) with p-DCB. The concentrations used were 96, 158, 173, 314 and 798 ppm (0.58, 0.95, 1.04, 2.05 and 4.8 mg/l, respectively). Exposure were conducted 7 hours/day, 5 days/week for 6-7 months. Adverse effects observed included liver and kidney histopathological changes with increased organ weights, pulmonary edema and congestion, splenic weight changes and reversible, non-specific eye changes.

It is difficult to reconcile the results of the previously-described studies with those of Varshavskaya (1968). She gave rats daily oral doses of 0.001, 0.01 or 0.1 mg/kg o-DCB in sunflower oil for nine months. No adverse effects were noted at the lowest dose, but varying degrees of inhibition of mitosis in the bone marrow, as well as neutropenia, abnormal conditioned reflexes and adrenal hypertrophy occurred at the two higher dose levels. While this study suggests that effects can be seen at very low doses when compared with other available studies, little in the way of experimental detail was presented in the publication. Therefore, it is difficult to assess fully the results presented, and one cannot conclude that these results should be used in risk characterization.

In the few reports available on studies of the carcinogenic potential of the DCBs in animals, the results are negative. One report of four cases of leukemia in humans attributed to o-DCB or a mixture of all three isomers has been published (Girard, et al., 1969). However, the possibility of exposure to other substances coincidentally cannot be ruled out. Hollingsworth, et al. (1956, 1958) exposed several species of animals to various oral and inhalation exposures of ortho- and para-dichlorobenzene for six to seven months. No pathological changes indicative of cancerous changes were observed.

Studies employing long-term or chronic exposures to o- and p-DCB were designed to evaluate the substances' chronic toxicity and carcinogenic potential. Preliminary assessment of the data from the NTP bioassay performed with o-DCB by gavage suggests that, under the conditions of the study, this substance is not a carcinogen in Fischer 344 rats or B6C3F1 mice (NTP, 1982). The NTP Board of Scientific Counselors added that no non-neoplastic lesions were noted in either the mice or the rats, suggesting that the maximum tolerated dose was not achieved. The results of the NTP gavage bioassay with p-DCB are not available to the Office of Drinking Water at this time.

A long-term inhalation study revealed no increase in tumor incidence or type following exposure to p-DCB in Alderley Park Wistar rats (Riley, et al., 1980a;summarized in Loeser and Litchfield,1983). At the high exposure level (500 ppm), changes indicative of non-neoplastic effects were observed: increases in liver, kidney, heart and lung weights (both sexes) and an increase in urinary protein and corproporphyrin output (in males).

Several teratogenicity studies have been conducted on two of the three isomers of dichlorobenzene. Studies were carried out in this country to evaluate the teratogenic potential of o-DCB in rats and rabbits (Dow, 1981; Hayes, et al., 1985). Hayes, et al. observed no teratogenic or fetotoxic effects in rat or rabbit fetuses whose dams were exposed to inhalation doses of o-DCB at levels up to 400 ppm. Similarly, no fetotoxic or teratogenic effects were noted in rabbits subjected to exposures of p-DCB at levels up to 500 ppm. In addition, results of a study conducted by Hodge, et al. (1977;summarized in Loeser and Litchfield, 1983) confirm the Hayes, et al. study in showing that maternal exposure to atmospheric levels of p-DCB up to 500 ppm on days 6-15 of pregnancy in the rat does not result in any embryotoxic, fetotoxic or teratogenic effects in the offspring.

Para-dichlorobenzene induces abnormal mitotic division in higher plants. Effects seen include shortening and thickening of chromosomes, precocious separation of chromatids, tetraploid cells, binucleate cells and chromosome bridges (c-mitosis) (Sharma and Battacharya, 1956; Sharma and Sarkar, 1957; Srivastava, 1966;

Gupta, 1972). Ortho-DCB was shown to produce abnormal mitotic division in the onion, *Allium cepa* (Ostergren and Levan, 1943).

Ortho-dichlorobenzene and para-dichlorobenzene were not mutagenic when tested in a culture of histidine-requiring mutants of *Salmonella typhimurium* or in the *E. coli* WP2 system (Anderson, *et al*., 1972; Anderson, 1976; Simmon, *et al*., 1979). However, all three isomers increased the frequency of back mutation of the methionine-requiring locus in the fungus, *Aspergillus nidulans* (Prasad and Pramer, 1968; Prasad, 1970). In addition, the meta isomer was shown to increase mitotic recombination in the *Saccharomyces cerevisiae* C3 yeast system (Simmon, *et al*., 1979). The results with the para isomer were ambiguous. These investigators also showed that both o- and m-DCB interacted with and damaged bacterial DNA in the *E. coli* W3110 polA^{+}/p3478 polA^{-} differential toxicity assay system.

No evidence of mutagenicity in animals has been published. Guerin, *et al*. (1971) showed that DCB did not produce a significantly different number of mitoses in rat lung cell cultures. Cytogenetic studies with rat bone marrow cells and a dominant lethal study in CD-1 mice following exposure to p-DCB were all negative (Anderson and Richardson, 1976; Anderson and Hodge, 1976).

II. GENERAL INFORMATION AND PROPERTIES

Chemical and Physical Properties

Three isomers of dichlorobenzene (DCB) exist: ortho (1,2-), meta (1,3-) and para (1,4-). Very little information is available on the meta isomer. Therefore, unless otherwise noted, the properties of this isomer will be assumed to be identical to those of the ortho isomer.

At room temperature (20-25°C), ortho- and meta-DCB are colorless neutral liquids; p-DCB is a colorless crystalline solid which readily sublimes (Irish, 1963). All isomers have a molecular weight of 147.01. Each is nearly insoluble in water, but readily soluble in many organic solvents, including ethanol, benzene and diethyl ether, as well as lipid. Freed, et al. (1979) determined the solubility of p-DCB in water at 25°C to be 79 ppm (79 mg/l). All isomers are heavier than water (specific gravity = 1.306, 1.288, 1.458 at 20°C for o-, m- and p-DCB, respectively). Thus, each would tend to sink in standing water. The ortho isomer has a vapor pressure of 1.56 mm Hg at 25°C. All three isomers are combustible.

In air, 1 ppm = 6.01 mg/m^3 and 1 mg/l = 166.3 ppm, at 25°C and 760 mm Hg (Irish, 1963).

Sato and Nakajima (1979) determined the water/air, blood/air, olive oil/air and olive oil/water partition coefficients for o- and m-DCB. These are listed in Table II-1. As the logs of their oil/water partition coefficients approach 4, both isomers appear are quite lipophilic. This is confirmed by the findings of Freed, et al. (1979) who established that p-DCB has a log P (n-octanol/water) of 3.38. It would be expected that these substances would tend to bioaccumulate in fatty tissues during prolonged, continuous exposures.

Table II-1. Partition Coefficients
(after Sato and Nakajima, 1979)

Partition Coefficient	Ortho-DCB	Meta-DCB
Water/Air (W)	9	5.5
Blood/Air (B)	423	201.4
Olive oil/Air (O)	39920	27080
Olive oil/Water (O/W)	4436	4924
Olive oil/Blood (O/B)	94	134
W·O	359280	148940
Log P (O/W)	3.65	3.69

The odor threshold for o-DCB in air is 2-4 ppm (AIHA, 1964). At 10-15 ppm, the smell becomes very noticeable, and at 25-30 ppm, it is considered unpleasant. Eye irritation becomes a problem at the same concentration range, while at exposures of 60-100 ppm, eye and mucous membrane irritation may be very painful. The odor threshold for p-DCB in air is 14-30 ppm in unacclimated persons (AIHA, 1964). Eye irritation begins at 50-80 ppm and becomes painful at 100 ppm. Kolle (1972) determined the odor threshold in water to range from 0.01-0.03 mg/l for the three DCB isomers.

Production and Use

o-Dichlorobenzene and p-dichlorobenzene are produced in considerable quantity. In 1981, the USITC reported that the production volume for o-DCB was 22,000 kkg and that for p-DCB, 34,000 kkg. At least 70 million pounds of p-DCB are used each year for moth control and as a space deodorant (Brown, et al., 1975). Apparently only two U.S. companies produce m-DCB (Ware and West, 1977). In 1974, 31,000 pounds were imported (USITC, 1974). Loss of o-DCB and p-DCB during their manufacture amounts to at least a million pounds a year for each isomer (Brown, et al., 1975). Meta-DCB is lost as a by-product of the manufacture of chlorobenzene. It also gets into the environment as a breakdown product of pesticides such as lindane.

The major uses of o-DCB are as a process solvent in the manufacture of toluene diisocyanate, and as an intermediate in the synthesis of dyestuffs, herbicides and degreasers (Ware and West, 1977). The bulk of p-DCB usage is in direct application as air deodorants and insecticides which account for 90% of its total consumption (Lowenheim and Moran, 1975; Ware and West, 1977). Use of o- and p-DCB as deodorizers in industrial wastewaters or in toilet bowl waters would suggest that there is a potential for these chemicals to be found in waters throughout the country in the future.

No documented uses of m-DCB were found in the literature.

III. TOXICOKINETICS

Absorption/Retention

The dichlorobenzenes are poorly soluble in water, but are highly lipid soluble (Neely, et al., 1974; Lu and Metcalf, 1975; Brown, et al., 1975). Thus, these substances can cross most of the barrier membranes, including those in the brain and placenta. Little information is available which demonstrates the percentage of a dose of DCB is absorbed and retained following exposure in any environmental medium. Yano (1979) administered 125 mg p-DCB/kg in oil to mice orally. He measured the carcass content of the compound (except for the gastrointestinal tract, hair, skin and tail) at 1,3,5,8 and 24 hours after dosing (see Figure III-1). The p-DCB tissue content reached maximum at 6 hours after dosing, falling to near zero after 8 hours. The author concluded that the absorption rate was 11 ± 2.9% per 24-hour period. The percentage of the dose absorbed was not identified in this study.

Based upon what is known about the absorption characteristics of benzene and the smaller chlorinated aliphatics (ethanes and ethylenes), it will be assumed that 100% of any oral dose of a dichlorobenzene is absorbed, while 30% of any DCB isomer inhaled over a period of one to several hours is absorbed and retained (Astrand, 1975;Dallas, et al., 1983).

Distribution

Kimura, et al. (1979) determined the distribution of p-DCB in tissues of fasted rats following a single oral dose of 200 mg/kg in oil. The highest concentration of p-DCB could be found in adipose tissue at all sampling intervals (1-120 hours post-

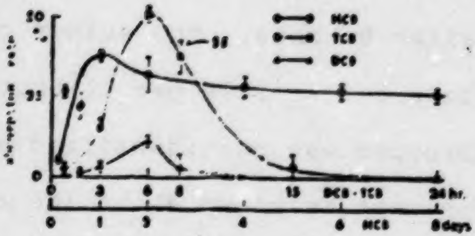


Figure III-1. Absorption rate of DCB, TCB and HCB in mice (whole body, except for the digestive tract, hair, skin and tail) after oral administration (DCB or TCB = 2.5mg/mouse;HCB=1.25 mg/mouse)

Source: Yano (1979)

exposure. Peak concentrations in fat occurred at 12 hours (>800 ug/g tissue). Kidney and liver showed the next highest levels of p-DCB, with peak concentrations occurring at 6-12 hours. Concentrations in the kidney reached about 32 ug/g, and those in the liver were 24 ug/g. Low, but quantifiable, levels of p-DCB also were found in blood, lung, heart and brain. Most of the p-DCB in the tissues except for adipose has disappeared within 48 hours after administration of the chemical. Levels of 14.1 ± 11.41 ug/g were detected in the adipose tissue after 120 hours.

Hawkins, et al. (1980) described the tissue distribution of p-DCB in adult female CFY rats following inhalation, oral or subcutaneous exposure. The animals received 10 consecutive daily exposures to p-dichloro (^{14}C) benzene either via inhalation at 1,000 ppm (~6,000 mg/m^3) for 3 hours/day or to a range of doses orally or subcutaneously. The compound was administered in sunflower oil at levels of 50, 125, 250, 375 or 500 mg/kg. The authors concluded that oral or subcutaneous doses of 250 mg/kg would yield concentrations in fat similar to those observed after the 1,000 ppm inhalation dose. However, as can be seen from the data in Table III-1, the levels in fat appear to be higher after the inhalation dose than after exposure by the other two routes.

Table III-1 shows tissue concentrations of radioactivity in animals killed 24 hours after two to ten consecutive daily doses. During inhalation exposure, concentrations reached their maxima after six days of exposure, remaining the same or falling slightly thereafter. At the maximum point, the highest levels were found in the fat, liver and kidney. Lung and muscle concentrations

Table III-1

Tissue concentrations of ^{14}C in female rats after daily atmospheric (inhal.) exposure (1,000 ppm) and oral or subcutaneous (s.c.) doses (250 mg/kg) of p-dichloro (^{14}C) benzene. Animals were killed at 24h after dosing and results expressed as ppm represent the mean of results from two animals.

No. of doses	Liver			Kidneys			Lungs			Muscle			Fat			Plasma		
	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.
2	14	11	21	24	27	30	9	7	18	5	5	9	418	218	372	12	13	26
4	22	18	22	40	29	32	12	13	12	6	6	4	579	369	302	12	14	16
6	28	14	24	43	23	47	11	10	14	7	<0.2	7	597	170	269	19	12	25
8	16	15	21	28	18	41	10	11	21	7	8	8	433	131	554	12	9	14
10	18	9	20	27	16	32	10	9	17	3	4	10	337	257	383	9	8	17

Source: Hawkins, et al. (1980).

reflected the levels in plasma. The highest tissue concentrations following the 250 mg/kg oral doses were reached after four doses. Again, the highest concentrations were found in liver, kidney and fat, with muscle and lung levels being similar to levels in plasma. After ten doses, all concentrations had fallen somewhat, with all but fat being similar to the plasma levels. Levels in fat remained very high. The pattern of distribution after subcutaneous injection of 250 mg/kg was similar to the patterns seen after oral or inhalation exposure.

Figure III-2 shows concentrations of radioactivity over a 24-hour period following cessation of exposure by inhalation. Except for the lungs (data not shown), concentrations were highest at 1 hour, falling thereafter. At 120 hours, the concentration in fat had fallen to about 5 ppm from 2,400 ppm at 1 hour. Concentrations in all other tissues were below the level of detection (0.2 ppm). Disappearance of radioactivity from six tissues was monitored in selected animals for up to 192 hours after cessation of exposure for 10 days by all three routes (Table IV-2). Concentrations declined rapidly at all sites to undetectable levels by 120 hours. After subcutaneous dosing, concentrations fell more slowly than with the other two routes of exposure, with detectable levels remaining after 192 hours.

Metabolism

Figures III-3-III-5 depict the metabolic pathways proposed for each of the three dichlorobenzene isomers. The figures reflect a composite of work reported in several studies.

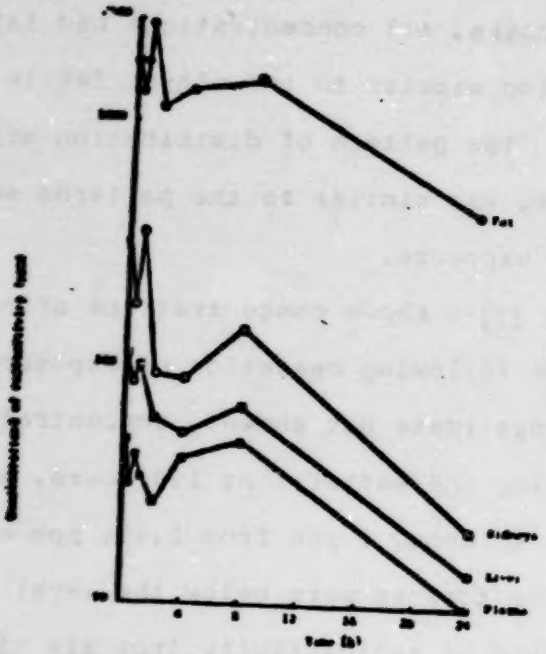


Figure III-2. Concentrations of radioactivity in the plasma and tissues of rats after repeated daily inhalation exposures of p-dichloro (^{14}C) benzene atmospheres for ten days.

Source: Hawkins, et al. (1980)

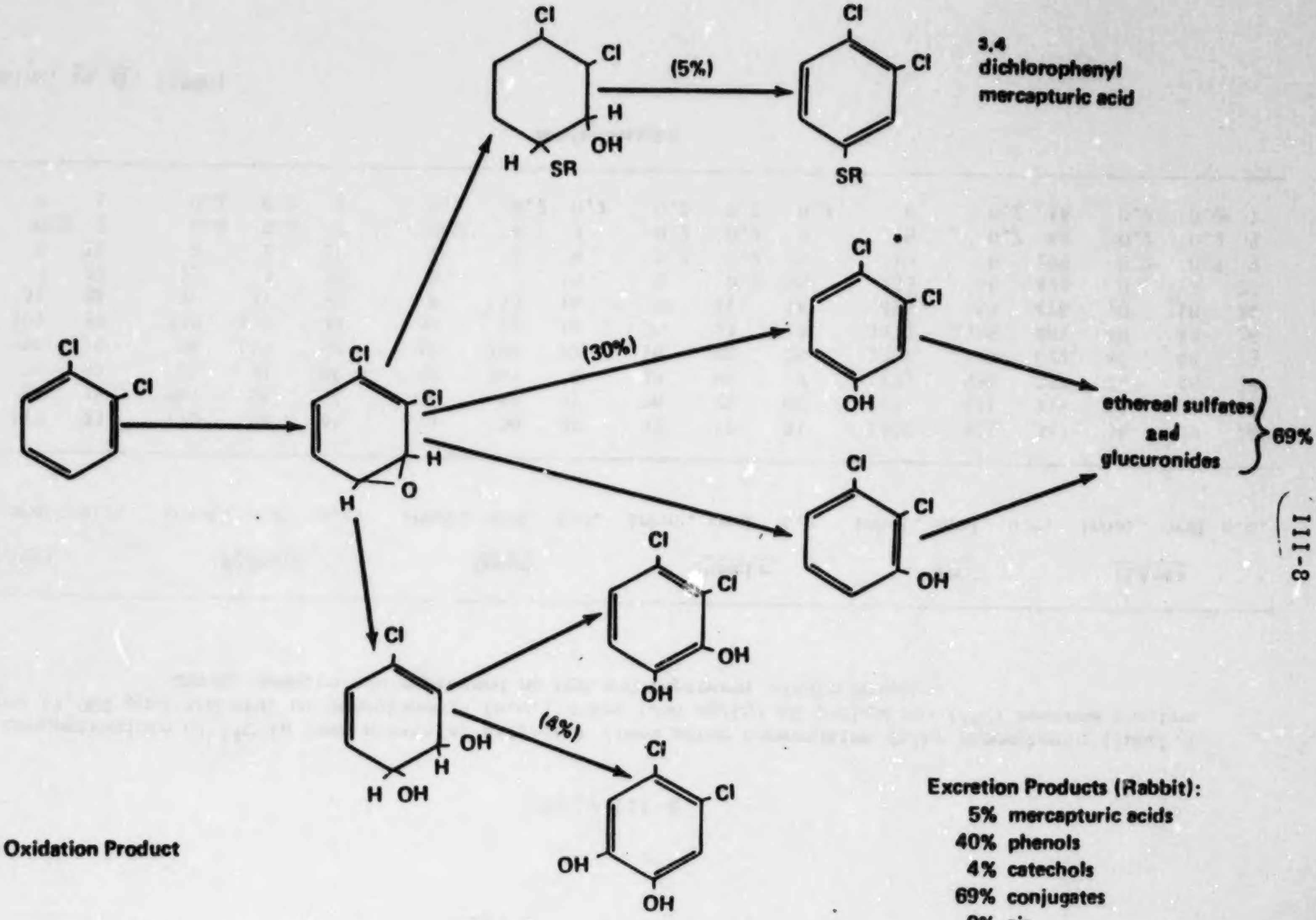
Table III-2

Tissue concentrations of ^{14}C in female rats at different times after consecutive daily atmospheric (inhal.) exposures (1,000 ppm) and oral or subcutaneous (s.c.) doses (250 mg/kg) of p-dichloro (^{14}C) benzene for ten days. Results are expressed as ppm and represent single animals.

Time of sacrifice (h)	Liver			Kidneys			Lungs			Muscle			Fat			Plasma		
	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.
0.5	83	117	23	172	74	45	178	58	24	43	12	11	1300	401	347	38	38	26
1	97	82	35	304	56	54	84	43	22	96	22	NS	2434	421	335	34	38	42
2	59	75	37	89	81	66	58	347	9	18	NS	9	1133	630	809	26	46	38
4	51	90	16	86	149	58	42	106	22	16	NS	25	1307	1423	622	40	48	29
8	66	101	30	138	123	34	39	75	18	35	23	18	1477	1385	481	48	43	26
24	14	31	28	21	31	31	9	13	18	3	11	16	425	559	476	10	18	24
48	16	7	22	15	3	38	8	3	15	5	0.2	NS	233	56	424	9	2	22
96	0.2	2	15	2	2	21	1	2	9	0.2	0.2	25	12	8	199	0.2	0.2	9
20	0.2	0.2	7	0.2	0.2	7	0.2	4	3	0.2	0.2	4	5	0.2	64	0.2	0.2	5
92	0.2	4	1	0.2	0.2	2	0.2	0.2	0.2	0.2	0.2	0.2	6	0.2	14	0.2	0.2	1

NS=No Sample

Source: Hawkins, et al. (1980)

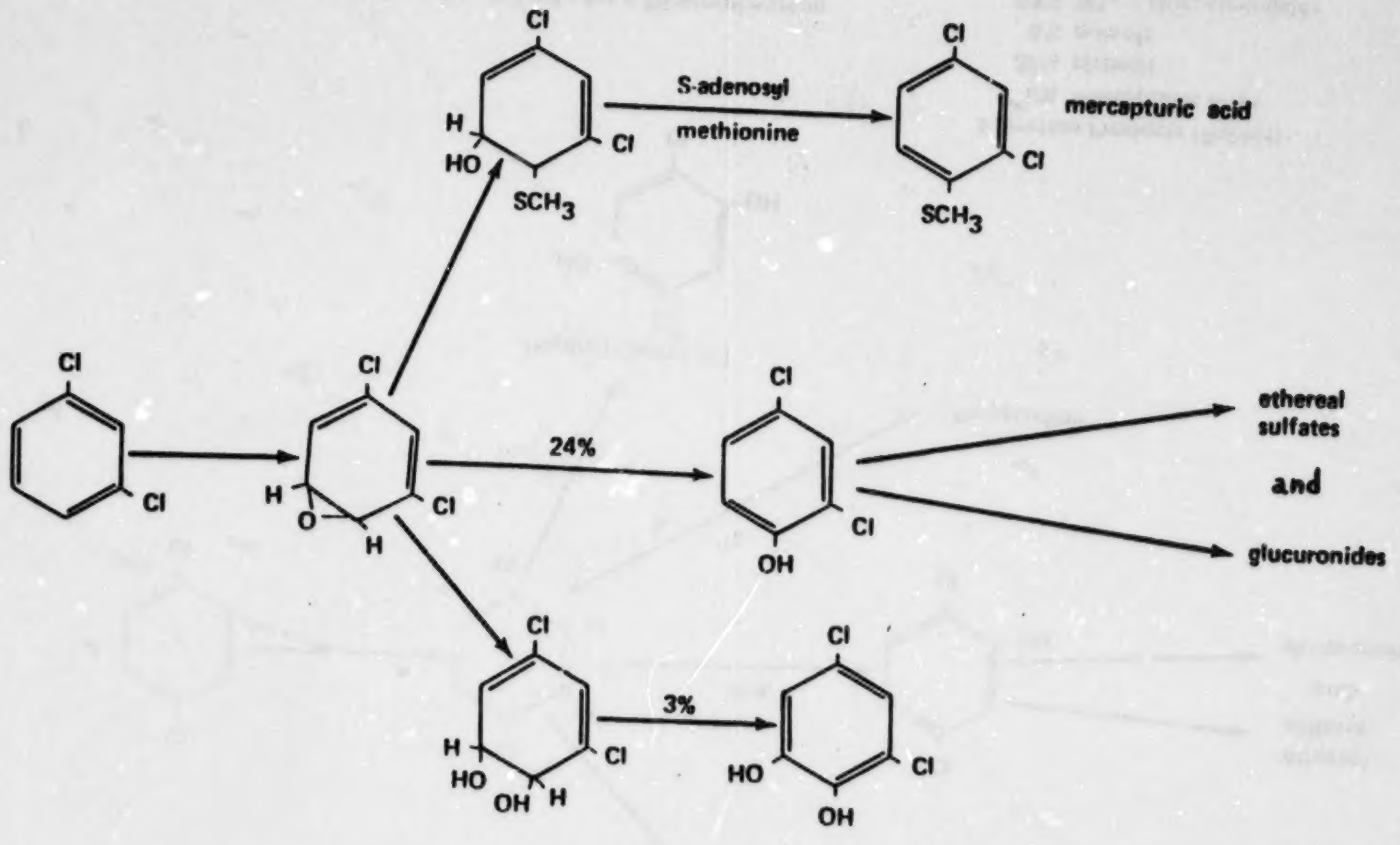

(5%)
(30%)
(4%)
Cl
H
OH
SR
O
*
3,4 dichlorophenyl mercapturic acid
ethereal sulfates and glucuronides
69%

*Main Oxidation Product

Excretion Products (Rabbit):

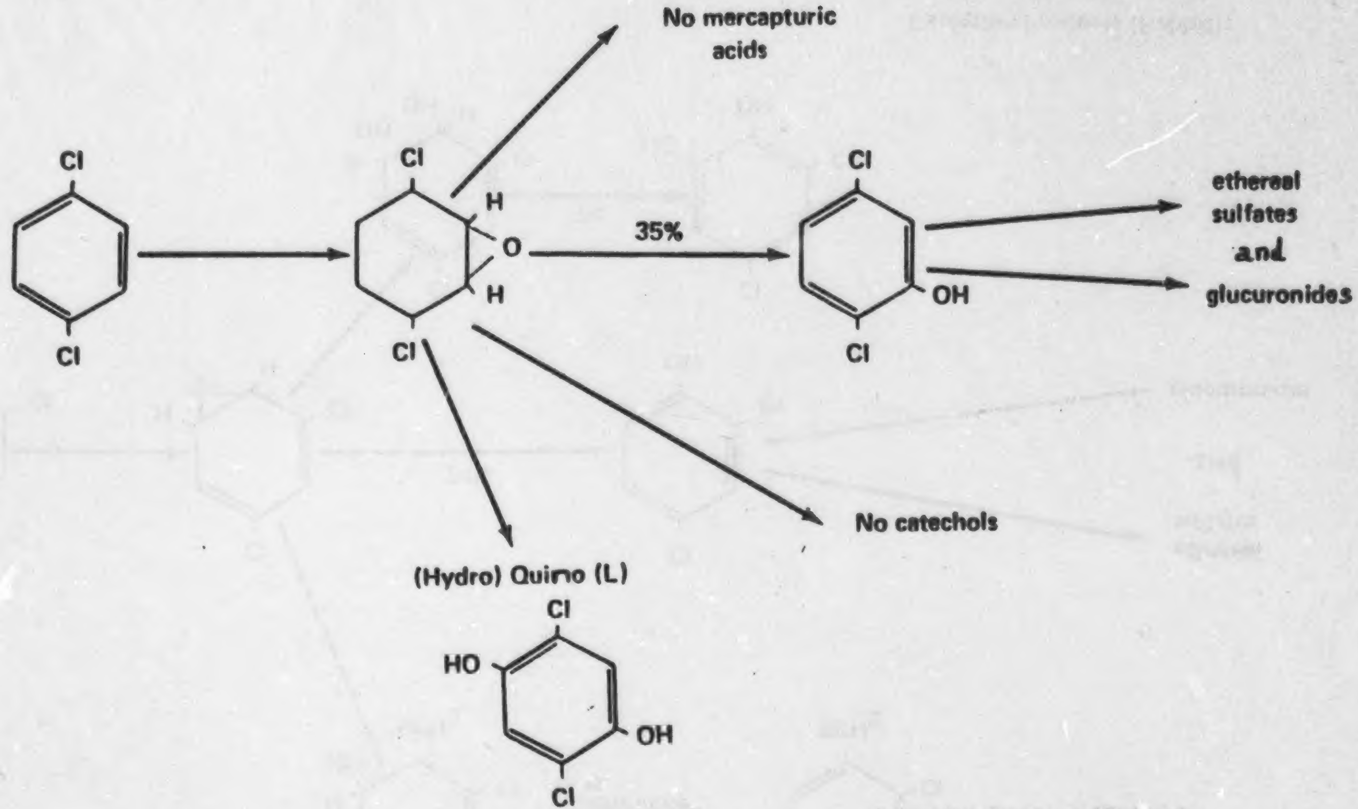
- 5% mercapturic acids
- 40% phenols
- 4% catechols
- 69% conjugates
- 0% air

Figure III-3. Proposed Metabolic Pathway for o-Dichlorobenzene


Cl
Cl
H
O
H
Cl
Cl
H
HO
SCH$_3$
Cl
Cl
S-adenosyl
methionine
Cl
Cl
SCH$_3$
mercapturic acid
24%
Cl
Cl
OH
ethereal
sulfates
and
glucuronides
H
HO
OH
H
Cl
Cl
3%
Cl
HO
OH
Cl

Excretion Products (Rabbit):
11% mercapturic acids
27% SO_3 + gluc.
25% phenols
3% catechols
0% air

FigureIII-4. Proposed Metabolic Pathway for m-Dichlorobenzene


No mercapturic acids
H
O
H
35%
Cl
OH
ethereal sulfates and glucuronides
No catechols
(Hydro) Quino (L)
HO
OH
Cl

Excretion Products (Rabbit):
0% mercapturic acids
35% phenols
6% quinols
63% SO_3 + gluc, conjugates
0% air

Figure III-5. Proposed Metabolic Pathway for p-Dichlorobenzene

In rabbits after oral administration (0.5 g/kg), all dichlorobenzenes were slowly metabolized by oxidation, mainly to dichlorophenols. The phenols and their conjugation products were excreted in five to six days. The major phenolic metabolite of o-DCB was 3,4-dichlorophenol (30% of the total dose), of m-DCB, 2,4-dichlorophenol (24% of the total dose) and of p-DCB, 2,5-dichlorophenol (35% of the total dose). About 3-11% of the ortho and meta isomers were excreted as dichlorocatechols. Para-DCB did not seem to form this metabolite. The ortho and meta isomers formed mercapturic acids (5-10% of the total), but p-DCB did not (Williams, 1959). The orientation of the mercapturic acid was the same as for the major phenolic product of each isomer. The phenolic metabolites were excreted as conjugates of glucuronic and sulfuric acid (Azouz, et al., 1953; Azouz, et al., 1955; Parke and Williams, 1955).

Kitamura, et al. (1977) studied the metabolism of meta- and para-dichlorobenzene in the mouse. After an intraperitoneal dose (level not stated) of either compound, urinary metabolites were characterized by GC-MS. The metabolites were the same as noted in Figures III-4 and III-5, with the exception that an unquantifie amount of unchanged compound also was detected.

Kimura, et al. (1979) identified, in addition to the already-characterized 3,5-dichlorophenol metabolite of p-dichlorobenzene, two sulfur-containing metabolites in tissues, blood and urine of rats following a single 200 mg/kg oral dose or a series of 800 mg/kg day doses over a week. These metabolites were identified as 2,5-dichlorophenyl methyl sulfoxide (M-1) and 2,4-dichlorophenyl methyl sulfone (M-2). Very small amounts of these two compounds were found (never more than 2 ug/g tissue). M-1 reached its highest concentration in the kidney, followed by blood (1.8 ug/g and 0.6 ug/g, respectively, at 12 hours post-exposure). Almost no M-1 was found in liver or adipose tissue. Concentrations in all tissues fell to near zero by 48 hours post-exposure.

M-2, on the other hand, was present in all four sampled tissues (blood>adipose>kidney>liver) at least until 120 hours post-exposure. Two peaks were observed in blood M-2 levels, at 18 and 48 hours after dosing, suggesting that this substance might undergo enterohepatic reabsorption and circulation.

The dichlorophenols appear to be the principal metabolic product of the DCB isomers in man. In a study of persons industrially-exposure to p-DCB, Pagnatto and Walkley (1965) used the urinary metabolite, 2,5-p-dichlorophenol, as a measure of exposure to p-DCB. In a case of accidental ingestion of an unknown quantity of p-DCB crystals by a three-year old boy, analysis of urine specimens yielded four abnormal phenols as well as 2,5-dichloroquinol. These were shown to be conjugated with glucuronic and sulfuric acids (Hallowell, 1959).

Hawkins, et al. (1980) studied the kinetics of excretion of ^{14}C-p-DCB in rats following the exposure conditions described in the Distribution section. Most of the ^{14}C activity (91-97%) was eliminated in the urine within five days after cessation of exposure, while small amounts were found in the feces and expired air. About 50-60% was excreted in the bile during the first two days, suggesting reabsorption in the enterohepatic circulation, just as Kimura et al.,

(1979) had postulated for the sulfur-containing metabolites. The major products excreted were identified by Hawkins, et al. (1980) as 2,5-dichlorophenol sulfate and, to a lesser degree, a glucuronide conjugate of the same phenol. Minor metabolites were dichloroquinol and a mercapturic acid of p-DCB.

Schmidt and Loeser (1977) previously had provided evidence for rapid elimination and lack of accumulation for p-DCB and its metabolites following termination of single or multiple exposures. These investigators administered p-DCB to rats a) as a single oral dose of 100 or 1000 mg/kg, 2) at 100 or 1000 ppm in the diet for 28 days, or 3) by inhalation over 76 weeks at concentrations of 75 or 500 ppm followed by a 36-week observation period.

In the acute study, all concentration curves showed rapid (2-4) decline in all tissues and plasma. About 40-60% of the dose was eliminated as 2,5-dichlorophenol conjugates in the urine. In the feeding study, concentrations in tissues declined from days 3-7, after which p-DCB maintained a steady state during the remaining three weeks. One week following cessation of exposure, neither parent p-DCB nor the 2,5-dichlorophenol metabolites were detectable in any tissue. During the inhalation study, tissue levels were lower after 76 weeks than after 26 weeks, demonstrating a decline to the end of the exposure. Neither p-DCB nor its major metabolite could be detected in fatty tissue 36 weeks after cessation of inhalation exposure.

Para-DCB has been shown to accumulate in human blood and adipose tissue. Morita and Ohi (1975) reported an average concentration of p-DCB in human adipose tissue of 2.3 ug/g (range = 0.2-11.7, N=34). Blood samples in six volunteers ranged from 4 ug/ml to 16 ug/ml and averaged 9.5 ug/ml. In another study, Morita, et al. (1975) reported levels of p-DCB in human adipose tissue of Tokyo residents. The concentration in the fat ranged from 0.02 ug/g to 9.9 ug/g, with a mean concentration of 1.7 ug/g. Since Morita and Ohi had established that there was a relatively high concentration of p-DCB in the atmosphere of the Tokyo metropolitan testing area (2.1-4.2 ug/m^3), they believed that inhalation of p-DCB was probably the major route of entry of the substance into the body.

In a study conducted in the New Orleans area, all three DCBs were detected in human blood samples (Dowty, et al., 1975). However, none of the chlorinated benzenes were detected in a 400 liter sample of air or in a sample of New Orleans drinking water, although many other organic compounds were confirmed. One might speculate that the individuals showing p-DCB in their blood might have been exposed to the substance during its use as a space deodorant or fumigant.

IV. HUMAN EXPOSURE

Humans may be exposed to dichlorobenzene in drinking water, food, and air. Detailed information concerning the occurrence of and exposure to dichlorobenzene in the environment is presented in another document entitled "Occurrence of Dichlorobenzenes in Drinking Water, Food, and Air" (Letkiewicz et al. 1983). This section summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food, and air.

Exposure Estimation

This analysis is limited to drinking water, food, and air, since these media are considered to be general sources common to all individuals. Some individuals may be exposed to dichlorobenzene from sources other than the three considered here, notably in occupational settings and from the use of consumer products containing dichlorobenzene. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and several factors over which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Unfortunately, data and methods to estimate exposure of identifiable population subgroups from all sources simultaneously have not yet been developed. To the extent possible, estimates are provided of the number of individuals exposed to each medium at various dichlorobenzene concentrations. The 70-kg male is used for estimating intake.

a. Water

Cumulative estimates of the U.S. populations exposed to various o- and p-dichlorobenzene levels in drinking water from public drinking water systems are presented in Tables IV-I and IV-II, respectively. The values in these tables were obtained using Federal Reporting Data Systems data on populations served by primary water supply systems (FRDS 1983) and the estimated number of these water systems that contain a given level of o- or p-dichlorobenzene.

Table IV-I. Total Estimated Cumulative Population (in Thousands) Exposed to o-Dichlorobenzene in Drinking Water Exceeding the Indicated Concentration

System type	Number of people served in U.S. (thousands)	Cumulative population (thousands) exposed to concentrations (ug/l) of: ≥0.5	>5
Groundwater	73,473	156	0
Surface water	140,946	1,431	0
Total	214,419	1,587	0
(% of total)	(100%)	(0.7%)	(0.0%)

Table IV-II. Total Estimated Cumulative Population (in Thousands) Exposed to p-Dichlorobenzene in Drinking Water Exceeding the Indicated Concentration

System type	Number of people served in U.S. (thousands)	Cumulative population (thousands) exposed to concentrations (ug/l) of: ≥0.5	>5
Groundwater	73,473	775	0
Surface water	140,946	859	0
Total	214,419	1,634	0
(% of total)	(100%)	(0.8%)	(0.0%)

An estimated 1,587,000 individuals (0.7% of the population of 214,419,000 using public water supplies) are exposed to levels of o-dichlorobenzene in drinking water at or above 0.5 ug/l. No individuals are estimated to be exposed to levels above 5 ug/l. Of the approximately 1.6 million people exposed to levels ranging from 0.5-5 ug/l, 1.4 million (90%) obtain water from surface water supplies.

An estimated 1,634,000 individuals (0.8% of the population using public supplies) are exposed to levels of p-dichlorobenzene in drinking water at or above 0.5 ug/l, while no individuals are estimated to be exposed to levels above 5 ug/l. Fifty-three percent of the approximately 1.6 million individuals exposed to levels ranging from 0.5-5 ug/l obtain water from surface water supplies, while 47% obtain water from groundwater supplies.

No individuals are estimated to be exposed to levels of m-dichlorobenzene above 0.5 ug/l.

No data were obtained on regional variations in the concentration of dichlorobenzene in drinking water. The highest concentrations are expected to occur near sites of production and use of dichlorobenzene.

Daily intake levels of o- and p-dichlorobenzene from drinking water were estimated using various exposure levels and the assumptions presented in Tables IV-III and IV-IV, respectively. The data suggest that the majority of the persons using public drinking water supplies would be exposed to intake levels for the dichlorobenzene isomers below 0.014 ug/kg/day.

Table IV-III. Estimated Drinking Water Intake of o-Dichlorobenzene

Exposure level (ug/l)	Persons using supplies exposed to indicated levels: Population	% of Total population	Intake (ug/kg/day)
>0.5	1,587,000	0.7%	>0.014
>5.0	0	0.0%	>0.14

Assumptions: 70-kg man, 2 liters of water/day.

Table IV-IV. Estimated Drinking Water Intake of p-Dichlorobenzene

Exposure level (ug/l)	Persons using supplies exposed to indicated levels: Population	% of Total population	Intake (ug/kg/day)
>0.5	1,634,000	0.8%	>0.014
>5.0	0	0.0%	>0.14

Assumptions: 70-kg man, 2 liters of water/day.

An indication of the overall exposure of the total population to dichlorobenzene can be obtained through the calculation of population-concentration values. These values are a summation of the individual levels of the dichlorobenzene isomers to which each member of the population is exposed. An explanation of the derivation of these values is presented in Appendix C.

Population-concentration estimates for o-dichlorobenzene in drinking water were 7.9×10^5 ug/l x persons (best case), 4.4×10^6 ug/l x persons (mean best case), 1.1×10^8 ug/l x persons (mean worst case), and 1.1×10^8 ug/l x persons (worst case). Assuming a consumption rate of 2 liters of water/day, population-exposure values of 1.6×10^6 ug/day x persons (best case), 8.8×10^6 ug/day x persons (mean best case), 2.2×10^8 ug/day x persons (mean worst case), and 2.2×10^8 ug/day x persons (worst case) were derived.

Population-concentration estimates obtained for m-dichlorobenzene were 0 (best case) and 1.1×10^8 (worst case). A median case value could not be calculated due to the absence of positive values in groundwater. Using these figures, population-exposure values of 0 ug/day x persons (best case) and 2.2×10^8 ug/day x persons (worst case) were derived.

Population-concentration estimates for p-dichlorobenzene in drinking water were 8.2×10^5 ug/l x persons (best case), 4.6×10^6 ug/l x persons (mean best case), 1.1×10^8 ug/l x persons (mean worst case), and 1.1×10^8 ug/l x persons (worst case). The population-exposure estimates derived from these values were 1.6×10^6 ug/day x persons (best case), 9.2×10^6 ug/day x persons (mean best case), 2.2×10^8 ug/day x persons (mean worst case), and 2.2×10^8 ug/day x persons (worst case).

b. Diet

Data on levels of dichlorobenzenes in foods in the United States were limited to concentrations of the chemicals in trout from the Great Lakes and in mother's milk. These data are insufficient for determining the intake of dichlorobenzene in the U.S. diet.

c. Air

Exposure to dichlorobenzene in the atmosphere varies from one location to another. The highest level of o-dichlorobenzene reported in the atmosphere was 19,000 ng/m^3 (19 ug/m^3) (Bozzelli and Kebbekus 1979 cited in Brodzinsky and Singh 1982). High levels, averaging greater than 1,000 ng/m^3 (1.0 ug/m^3), have been detected in other areas. Normal levels, however, are somewhat lower. Brodzinsky and Singh (1982) calculated median air levels of o-dichlorobenzene for rural/remote areas, urban/suburban areas, and source dominated areas of 0.0 ng/m^3 (0.0 ug/m^3), 6.6 ng/m^3 (0.0066 ug/m^3), and 350 ng/m^3 (0.35 ug/m^3), respectively.

The highest level of m-dichlorobenzene reported in the atmosphere was 16,000 ng/m^3 (16 ug/m^3) (Wallace 1981 cited in Brodzinsky and Singh 1982). Average levels greater than 1,500 ng/m^3 (1.5 ug/m^3) have been reported in other areas. The following median concentrations were calculated for m-dichlorobenzene: rural/remote areas, 0.0 ng/m^3 (0.0 ug/m^3); urban/suburban areas, 36 ng/m^3 (0.036 ug/m^3); and source dominated areas, 560 ng/m^3 (0.56 ug/m^3).

The maximum level reported for p-dichlorobenzene in the atmosphere was 60,000 ng/m^3 (60 ug/m^3) (Bozzelli and Kebbekus 1979 cited in Brodzinsky and Singh 1982). Mean levels of p-dichlorobenzene above 1,000 ng/m^3 (1 ug/m^3) were found in two locations. Median air levels of p-dichlorobenzene for rural/remote areas, urban/suburban areas, and source dominated areas were 0.0 ng/m^3 (0.0 ug/m^3), 280 ng/m^3 (0.28 ug/m^3), and 0.0 ng/m^3 (0.0 ug/m^3), respectively.

The monitoring data available are not sufficient to determine regional variations in exposure levels for the dichlorobenzenes. However, urban and industrial areas generally appear to contain higher levels, as expected.

The daily respiratory intake of each of the isomers of dichlorobenzene was estimated using the assumptions presented in Tables IV-V through IV-VII and the median and maximum levels for the dichlorobenzenes reported above. The estimates in Tables IV-V and IV-VI indicate that the daily intake of o- and m-dichlorobenzene from air for adults in source dominated areas is approximately 0.1 and 0.2 ug/kg/day, respectively. A similar value (0.09 ug/kg/day) was calculated for p-dichlorobenzene in urban/suburban areas (Table IV-VII). In contrast, the intakes calculated using the maximum o-, m-, and p-dichlorobenzene levels reported are 6.3, 5.3, and 20 ug/kg/day, respectively; few if any persons are believed to be exposed at those levels. The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

Table IV-V. Estimated Respiratory Intake of o-Dichlorobenzene

Exposure (ug/m^3)	Intake (ug/kg/day)
Rural/remote (0.0)	0.0
Urban/suburban (0.0066)	0.0022
Source dominated (0.35)	0.12
Maximum (19)	6.2

Assumptions: 70-kg man, 23 m^3 of air inhaled/day (ICRP 1975).

Table IV-VI. Estimated Respiratory Intake of m-Dichlorobenzene

Exposure (ug/m^3)	Intake (ug/kg/day)
Rural/remote (0.0)	0.0
Urban/suburban (0.036)	0.012
Source dominated (0.56)	0.18
Maximum (16)	5.3

Assumptions: 70-kg man, 23 m^3 of air inhaled/day (ICRP 1975).

Table IV-VII. Estimated Respiratory Intake of p-Dichlorobenzene

Exposure (ug/m^3)	Intake (ug/kg/day)
Rural/remote (0.0) Source dominated (0.0)[a]	0.0
Urban/suburban (0.28)	0.092
Maximum (60)	20

[a] Value reported for source dominated areas was lower than that reported for urban/suburban areas.

Assumptions: 70-kg man, 23 m^3 of air inhaled/day (ICRP 1975).

In addition to the available monitoring data, Systems Applications (1982) has provided estimates of atmospheric levels of o- and p-dichlorobenzene by applying air dispersion models to dichlorobenzene emission sources. The computed average concentrations of the dichlorobenzenes and the number of individuals estimated to be exposed to these concentrations are presented in Tables IV-VIII and IV-IX. Specific point sources in these tables are individually identified sources with known locations and modes and rates of emissions. These are generally manufacturing plants. General point sources are sources that are too numerous, small, or of uncertain location to be treated individually. However, these sources produce isolated patterns of significant concentration. Area sources are sources that are numerous and emit only small concentrations of the chemical (e.g., home chimneys, automobiles). The estimates presented for o-dichlorobenzene in Table IV-VIII suggest that only a small number of individuals (less than 700,000) are exposed to o-dichlorobenzene concentrations greater than 250 ng/m^3 (0.25 ug/m^3). Estimates for p-dichlorobenzene (Table IV-IX) suggest that 62,000,000 individuals are exposed to p-dichlorobenzene levels at or above 250 ng/m^3 (0.25 ug/m^3).

Tables IV-VIII and IV-IX also present total population-concentration estimates for o- and p-dichlorobenzene (6.45×10^6 and 5.14×10^7 ug/m^3 x persons, respectively). Assuming an inhalation rate of 23 m^3 of air/day, population-exposures of 1.48×10^8 and 1.18×10^9 ug/day x persons, respectively, were calculated.

SUMMARY

Tables IV-X, IV-XI, and IV-XII present a general view of the total amount of o-, m-, and p-dichlorobenzene, respectively, received by an adult male from air and drinking water. Insufficient data were obtained on levels of dichlorobenzene in foods to assess the relative intake from that source.

The data presented have been selected from an infinite number of possible combinations of concentrations for the two sources. The actual exposures encountered would represent some finite subset of this infinite series of combinations. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to dichlorobenzene at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

Table IV-VIII. Exposure and Dosage Summary for Airborne o-Dichlorobenzene

Concentration level (ug/m^3)	Population exposed (persons)				Dosage (ug/m^3 x persons)			
	Specific point source	General point source	Area source	U.S. total	Specific point source	General point source	Area source	U.S. total
50	2	0	0	2	91	0	0	91
25	25	0	0	25	892	0	0	892
10	234	0	0	234	3,610	0	0	3,610
5	917	0	0	917	8,410	0	0	8,410
2.5	5,406	0	0	5,406	23,600	0	0	23,600
1	36,787	0	0	36,787	69,500	0	0	69,500
0.5	93,389	0	0	93,389	110,000	0	0	110,000
0.25	172,270	0	505,140	677,410	137,000	0	232,451	369,451
0.1	426,427	0	9,149,730	9,576,157	178,000	0	1,772,052	1,950,052
0.05	626,291	0	33,072,205	33,698,495	192,000	0	3,479,775	3,671,775
0.025	839,531	0	81,759,648	82,599,179	200,000	0	5,056,481	5,256,481
0.01	1,525,505	800	142,928,535	144,454,840	210,000	9	6,121,131	6,331,140
0	6,113,449	--	158,679,135	--	224,000	2,460	6,225,594	6,452,054

Note: The use of "--" as an entry indicates that the incremental increase in the population exposed or the dosage is not significant (relative to the last entry in that column or to an entry in another column at the same row) or that the exposure of the same population may be counted in another column.

Source: Systems Applications 1982

Table IV-IX. Exposure and Dosage Summary for Airborne p-Dichlorobenzene

Concentration level (ug/m^3)	Population exposed (persons)				Dosage (ug/m^3 x persons)			
	Specific point source	General point source	Area source	U.S. total	Specific point source	General point source	Area source	U.S. total
50	2	0	0	2	118	0	0	118
25	8	0	0	8	328	0	0	328
10	42	0	0	42	815	0	0	815
5	127	0	0	127	1,420	0	0	1,420
2.5	389	0	505,140	505,529	2,350	0	1,917,818	1,920,168
1	1,691	0	9,149,730	9,151,421	4,330	0	14,620,149	14,624,479
0.5	3,879	0	26,976,292	26,980,171	5,930	0	26,029,918	26,035,848
0.25	10,792	0	61,583,693	61,594,485	8,400	0	37,167,988	37,176,388
0.1	36,631	0	--	--	12,200	0	49,590,816	49,603,016
0.05	126,422	0	--	--	18,400	0	--	--
0.025	384,501	0	--	--	27,400	0	--	--
0.01	888,210	3,400	--	--	35,200	50	--	--
0.005	--	19,600	--	--	--	160	--	--
0	2,341,084	--	158,679,135	--	41,400	3,360	51,363,678	51,408,438

Note: The use of "--" as an entry indicates that the incremental increase in the population exposed or the dosage is not significant (relative to the last entry in that column or to an entry in another column at the same row) or that the exposure of the same population may be counted in another column.

Source: Systems Applications 1982

Table IV-X. Estimated Intake of o-Dichlorobenzene from the Environment by Adult Males in ug/kg/day (% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air			
	Rural/remote (0.0 ug/m^3)	Urban/suburban (0.0066 ug/m^3)	Source dominated (0.35 ug/m^3)	Maximum (19 ug/m^3)
0	0.0 (--)	0.0022 (0%)	0.12 (0%)	6.2 (0%)
0.5[a]	0.014 (100%)	0.016 (88%)	0.13 (11%)	6.2 (0.2%)
5.0[b]	0.14 (100%)	0.14 (100%)	0.26 (54%)	6.3 (2.2%)

Intake from each source (see Sections 5.1-5.3):

Water:	0.5 ug/l:	0.014 ug/kg/day
	5.0 ug/l:	0.14 ug/kg/day
Air:	0.0 ug/m^3:	0.0 ug/kg/day
	0.0066 ug/m^3:	0.0022 ug/kg/day
	0.35 ug/m^3:	0.12 ug/kg/day
	19 ug/m^3:	6.2 ug/kg/day
Food:	Not included	

[a] 1,587,000 individuals using public drinking water systems are estimated to be exposed to levels ≥ 0.5 ug/l (0.7% of population using public water supplies).

[b] No individuals using public drinking water systems are estimated to be exposed to levels > 5.0 ug/l.

Table IV-XI. Estimated Intake of m-Dichlorobenzene from the Environment by Adult Males in ug/kg/day (% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air			
	Rural/remote (0.0 ug/m^3)	Urban/suburban (0.036 ug/m^3)	Source dominated (0.56 ug/m^3)	Maximum (16 ug/m^3)
0	0.0 (--)	0.012 (0%)	0.18 (0%)	5.3 (0%)
0.5[a]	0.014 (100%)	0.026 (54%)	0.19 (7.4%)	5.3 (0.3%)

Intake from each source (see Sections 5.1-5.3):

Water:	0.5 ug/l:	0.014 ug/kg/day
Air:	0.0 ug/m^3:	0.0 ug/kg/day
	0.036 ug/m^3:	0.012 ug/kg/day
	0.56 ug/m^3:	0.18 ug/kg/day
	16 ug/m^3:	5.3 ug/kg/day
Food:	Not included	

[a] No individuals using public drinking water systems are estimated to be exposed to levels ≥ 0.5 ug/l.

Table IV-XII. Estimated Intake of p-Dichlorobenzene from the Environment by Adult Males in ug/kg/day (% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air		
	Rural/remote Source dominated (0.0 ug/m^3)	Urban/suburban (0.28 ug/m^3)	Maximum (19 ug/m^3)
0	0.0 (--)	0.92 (0%)	20 (0%)
0.5[a]	0.014 (100%)	0.11 (13%)	20 (0.07%)
5.0[b]	0.14 (100%)	0.23 (61%)	20 (0.7%)

Intake from each source (see Sections 5.1-5.3):

Water: 0.5 ug/l: 0.014 ug/kg/day
5.0 ug/l: 0.14 ug/kg/day

Air: 0.0 ug/m^3: 0.0 ug/kg/day
0.28 ug/m^3: 0.092 ug/kg/day
60 ug/m^3: 20 ug/kg/day

Food: Not included

[a] 1,634,000 individuals using public drinking water systems are estimated to be exposed to levels $\geq$ 0.5 ug/l (0.8% of population using public water supplies).

[b] No individuals using public drinking water systems are estimated to be exposed to levels > 5.0 ug/l.

Brodzinsky and Singh (1982) calculated median urban/suburban air levels of o-, m-, and p-dichlorobenzene of 0.0066, 0.036, and 0.28 ug/m^3, respectively, based on air monitoring data. Assuming those air levels, drinking water would be the predominant source of exposure in the adult male at drinking water levels above 0.08, 0.42, and 3.2 ug/l, respectively. An accurate assessment of the number of individuals for which drinking water is the predominant source of exposure cannot be determined from the data since specific locations containing high concentrations of the dichlorobenzenes in drinking water and low concentrations of the dichlorobenzenes in ambient air and food are unknown.

Population-exposure estimates for o- and p-dichlorobenzene in drinking water and air were reported previously. Estimates for o-dichlorobenzene in drinking water ranged from 0.016-2.2 x 10^8 ug/day x persons; the estimate for ambient air was 1.48 x 10^8 ug/day x persons. These population-exposures are comparable. Estimates for p-dichlorobenzene in drinking water also ranged from 0.016-2.2 x 10^8 ug/day x persons; the estimate for ambient air was 1.18 x 10^9 ug/day x persons. These estimates suggest that ambient air may be a slightly greater source of exposure to p-dichlorobenzene than drinking water on a general population basis. Comparison of these estimates, however, may be deceiving since the same population-exposure level can occur if: 1) a whole population is exposed to moderate levels of a chemical or 2) some segments of the same population are exposed to high levels and others to low levels. The population-exposure values presented give no indication of the relative predominance of drinking water and air as specific sources of o- and p-dichlorobenzene on a site-by-site or subpopulation basis.

The relative source contribution data are based on estimated intake and do not account for a possible differential absorption rate for dichlorobenzene by route of exposure. The relative dose received may vary from the relative intake. In addition, the relative effects of the chemical on the body may vary by different routes of exposure.

V. HEALTH EFFECTS IN ANIMALS

The acute and chronic toxicities of the dichlorobenzenes closely parallel those observed with chlorobenzene. A search of the literature uncovered no data on the acute or chronic toxic effects on m-DCB. However, a number of studies have been published on the acute and long-term effects of the other two dichlorobenzene isomers. In general, p-DCB found to be less toxic than the ortho-isomer.

The dichlorobenzenes produce sedation and anesthesia after acute, high level exposures. Acute poisoning is characterized by signs of disturbance of the central nervous system (CNS). There may be hyperexcitability, restlessness, muscle spasms or tremors followed by varying degrees of CNS depression. The most frequent cause of death is respiratory depression. Acute exposure at very high levels also may result in liver or kidney damage.

Acute Exposure

Several investigators have determined the acute lethal dose levels after exposure to the dichlorobenzenes in several species. These data are presented in Table V-1.

Varshavskaya (1968), in her comparative studies on the adverse effects of the lower chlorinated benzenes, showed that, in the acute exposures, that o-DCB was slightly less toxic in mice and rats than chlorobenzene (CB), and that p-DCB was even less toxic than o-DCB or CB. o-DCB was slightly more toxic than CB in rabbits and guinea pigs.

Intravenous doses of 0.25-0.5 cc/kg (0.33-0.66 g/kg) body weight were fatal to rabbits within 24 hours; doses of 1 cc/kg (1.31 g/kg) were fatal within 20 seconds (Cameron, et al., 1937).

Table V-1

Acute Toxicity Data for o- and p-Dichlorobenzene

Animal	Route	LD_{50}	LCL_0	Reference
o-Dichlorobenzene				
Rat	Oral	500 mg/kg		NIOSH, 1978
Guinea Pig	Oral	2000 mg/kg		Hollingsworth, et al., 1958
Rat	Inhal.		821 ppm/7 hr	Hollingsworth, 1958
Guinea Pig	Inhal.		800 ppm/7 hr	Hollingsworth, 1958
Guinea Pig	Inhal.		800 ppm/24 hr	Cameron, et al., 1937
p-Dichlorobenzene				
Rat	Oral	500 mg/kg		NIOSH, 1978
Rat	Oral	2500 mg/kg		Hollingsworth, et al., 1956
Guinea Pig	Oral	2800 mg/kg (LDL_O)		Hollingsworth, et al., 1956
Mouse	SC	5145 mg/kg		Irie, et al., 1973

In the mouse, the LD_{50} value for a subcutaneous dose of p-DCB was found to be 5,145 mg/kg (Irie, et al., 1973).

Inhalation of 800 ppm (4,800 mg/m^3) o-DCB by rats for 11-50 hours was irritating to the eyes and nose, produced slight changes in the tubular epithelium of the kidney and resulted in confluent necrosis of the liver (Cameron, et al., 1937).

Rabbits, rats and guinea pigs exposed for 20-30 minutes daily to 100 mg p-DCB/l of air for 5-9 days showed marked irritation of the eyes and nose, muscle twitching, tremors, CNS depression, nystagmus and rapid but labored breathing (Zupko and Edwards, 1949). The animals recovered within 30-180 minutes after being removed from the p-DCB-rich atmosphere. A definite granulocytopenia was observed in 11 rabbits; a questionable change was seen in three others and no change was found in the remaining three. Body weight decreased in 14 animals; three rabbits showed an increase. In 13 rats, CNS depression was observed to be greater than in rabbits. A definite granulocytopenia was observed in eight rats, while a questionable change was seen in three, and no change was found in two others. Nine rats showed a decrease in total white cell count, while four showed an increase. In addition to exhibiting similar symptoms to p-DCB inhalation as rats and rabbits, guinea pigs also exhibited granulocytopenia in most cases. Five animals showed a frank decrease in granulocytes, two showed a tendency toward lowering, while two others showed no decrease. Six of the nine animals suffered a weight loss, while three did not.

When the animals were removed from exposure to p-DCB, the decrease in granulocytes was reversed with the granulocyte level returning to normal within three to four weeks.

Several animal studies document the effects of o- and p-DCB on the eye and/or skin. The results are summarized in Table V-2.

Yang, et al. (1979) showed that both o- and p-DCB alter pancreatic function. They administered a single intraperitoneal dose of each substance in sesame oil (5 mmol/kg: 735 mg/kg) to fasted adult male Holtzman rats. Control animals received an equivalent volume of sesame oil (1 ml/kg). Experimental measurements were made 24 hours later. After treatment with o-DCB, bile duct-pancreatic flow (BDPF) was increased nearly ten-fold over that observed in control animals ($P<0.05$). p-DCB did not alter the flow significantly. Protein concentration of the bile effluent was reduced to about 25% of controls after o-DCB ($P<0.05$), but was not changed after p-DCB. However, p-DCB did increase significantly the chloride content of the effluent, whereas o-DCB did not. Neither compound affected the rate of bile flow or serum levels of GPT. The authors could not offer as explanation of the mechanism by which the observed changes occurred, although they did conclude that the mechanism did not involve secretin or cholinergic stimulation of the pancreas. Thus, the significance of these findings remains unknown.

Effects on Prophyrin Metabolism

All of the chlorinated benzenes have been shown to produce disturbances in prophyrin metabolism, although they vary in potency, usually increasing in potency with increasing numbers of chlorines.

Rimington and Ziegler (1963) showed that administration of 500 to 1200 mg/kg of each chlorinated benzene (except pentachloro-

Table V-2

Effects of Chlorinated Benzenes on the Eye and Skin (modified from Ware and West, 1977)

Chemical	Animal	Exposure	Effects	Reference
o-Dichlorobenzene	Rabbits	2 drops in each eye	Pain slight. Conjunctival irritation. Cleared in 7 days	Hollingsworth, et al., 1958
p-Dichlorobenzene	Rabbits	4.6 to 4.8 mg/l (770-800 ppm) for 8 hours, inhalation	Lateral nystagmus, transitory edema of cornea. - edema of optic nerve. Eye changes reversible (17 days). No lens changes or deposits in the vitreous.	Pike, 1944
p-Dichlorobenzene	Rabbits	Inhalation. 5 gm vaporized/2 days 5-47 days	No opacity of the lens (liver necrosis and death)	Berliner, 1939
p-Dichlorobenzene	Rabbits	Oral, 5 gms p-DCB daily for 3 weeks	Opacity of lens slight and mod	Berliner, 1939

chlorobenzene) to male albino rats for 5-15 days leads to a hepatic porphyria characterized by elevated levels of precursor porphyrins in liver and feces. The investigators administered o-DCB in liquid paraffin at maximum dose levels of 455 mg/kg/day for 15 days, and p-DCB, also in liquid paraffin, at maximum dose levels of 770 mg/kg/day for 5 days. The doses were gradually increased until a dosage level was reached which yielded high porphyrin excretion, but few fatalities. The first signs of intoxication were an increase in urinary coproporphyrin and porphobilinogen (PBG). An increase in aminolevulinic acid excretion was a late effect. The most potent compound was p-dichlorobenzene (see Table V-3). Mean peak values of urinary coproporphyrin increased 10-15 fold after p-DCB to about 62 ug/day when compared with control. o-DCB treatment elicited a 6-10 fold increase in the same parameter (to about 43 ug/day). After p-DCB, a nearly 100-fold increase in urinary uroporphyrin levels occurred, while o-DCB caused an increase of about 10-fold. Porphobilinogen levels measured 1328 ug/day after p-DCB, but only 26.65 ug/day after o-DCB, increasing from a control level of 2.5-6.5 ug/day. After p-DCB, a 10-fold increase (to 437 ug/day) in Δ-ALA levels were observed, while after o-DCB, levels actually decreased to 11.3 ug/day (normal range = 38.7-51.6 ug/day.)

Several parameters of liver function also were measured by Rimington and Ziegler (Table V-4). In contrast to the change observed in urinary coprophyrin excretion, liver coproporphyrin was unchanged after p-DCB, and roughly doubled after o-DCB (to 10.05 ug/100g tissue vs. 4.5 ug/100g in the controls). Protoporphyrin levels increased six-fold after p-DCB (60.5 ug/100g), but only

Table V-3

Mean Peak Values of Urinary Porphyrin Precursors Following Treatment
With Maximum Doses Tested for each Chlorinated Benzene
(Rimington and Ziegler, 1963)

No. of rats	Compound	Sol-vent	Max. dose (mg/kg)	Days on max. dose	Copro-porphyrin (ug/day)	Uropor-phyrin (ug/day)	PBG (ug/day)	ALA (ug/day)	ALA/PRG
	Controls*	C			4.3-6.8	0.1-0.3	2.5-6.5	38.7-51.6	
3	Monochlorobenzene	P	1140	5	30.50	1.40	26.70	56.40	2.98
3	1,2-Dichlorobenzene	P	455	15	43.10	2.01	26.65	11.32	2.11
3	1,4-Dichlorobenzene	P	770	5	61.80	10.99	1328.10	437.41	0.36
3	1,2,3-Trichlorobenzene	C	785	7	36.59	0.72	57.38	36.26	1.01
3	1,2,4-Trichlorobenzene	C	730	15	58.31	2.73	179.00	145.70	1.06
3	1,2,3,4-Tetrachloro-benzene	P	660	10	28.96	3.80	520.63	315.78	0.61
6	1,2,4,5-Tetrachloro-benzene	C	905	5	4.10	0.22	6.50	18.08	6.69

P = liquid paraffin; C= 1% cellofas
PBG = porphobilinogen; ALA = ∠ -aminolevulinic acid.
* Mean max. and min. of 5 rats during 41 days.

Table V-4

Porphyrins, Porphobilinogen and Catalase Activity In Livers of Rats Treated With Chlorinated Benzenes (Rimington and Ziegler, 1963)

No. of rats	Compound	Max. dose (mg/kg)	Days on max. dose	Copro-porphyrin (ug/100 day)	Protopor-phyrin (ug/100 g)	Uropor-phyrin (ug/100 g uncorrected)	PBG	Catalase (meq/mg) wet wt.
2	Controls		.	4.5	9.7	1.3	—	0.85
2	Monochlorobenzene	1400	5	Trace	13.00	6.35	—	0.502
2	1,2-Dichlorobenzene	450	15	10.05	34.80	14.40	—	0.364
2	1,4-Dichlorobenzene	770	5	5.07	60.35	60.35	—	0.880
2	1,2,3-Trichlorobenzene	780	7	2.65	3.55	20.00	—	0.857
5	1,2,4-Trichlorobenzene	500	10	45.56	55.60	52.70	++	0.747
3	1,2,3,4-Tetrachlorobenzene	660	10	35.04	56.57	41.32	+	0.772
2	1,2,4,5-Tetrachlorobenzene	850	5	6.30	9.90	2.15	—	0.838

3.5-fold after o-DCB (to 34.8 ug/100g from a control level of 9.7 ug/100g). Catalase levels were unaffected by p-DCB, but more than halved after o-DCB (0.85 meq/mg wet weight for control to 0.364 meq/mg wet weight after p-DCB exposure). These decreases in catalase activity were observed only in animals in which marked histological changes indicative of severe liver damage (i.e., large areas of necrosis) were observed. Thus, hepatotoxic changes occurred in o-DCB-treated animals, but not in those treated with p-DCB. In addition, p-DCB did not alter the glutathione content of the liver. o-DCB was not tested. From these data, the authors suggested that the mechanisms which produce porphyria derangements may be different from those which lead to liver necrosis.

Carlson (1977) studied chlorinated benzene induction of liver porphyria in groups of five female rats receiving p-DCB, 1,2,4-trichlorobenzene or hexachlorobenzene. Each substance was suspended in corn oil and administered orally at 0, 50, 100 or 200 mg/kg/day for 30, 60, 90 or 120 days. After the last dose, the animals were placed into metabolism cages for collection of 24-hour urine samples. At the end of the 24-hour period, the animals were sacrificed and liver and urinary porphyrins measured. The results after exposure to p-DCB can be seen in Table V-5. The data show that this substance has little potential for causing porphyria at these doses in female rats. After 30 and 60 days, liver weight increased in a dose-dependent manner. Even after 120 days, only slight increases in liver porphyrins occurred. Urinary excretion of Δ-ALA, porphobilinogen (PBG) or porphyrins was not increased over control levels.

Table V-5

Effect of 1,4-Dichlorobenzene p.o. on Porphyrin Production and Excretion in Female Rats.

(Carlson, 1977)

Dose (mg/kg)	Liver wt(g)	Liver porphyrins (ng/g)	Urine porphyrins (ug/24h)
	30 days of administration		
0	6.8 ± 0.3^{a}	246 ± 21^{a}	1.5 ± 0.2^{a}
50	6.6 ± 0.4^{a}	269 ± 20^{a}	1.9 ± 0.3^{a}
100	7.0 ± 0.2^{a}	251 ± 22^{a}	1.4 ± 0.2^{a}
200	8.0 ± 0.3^{b}	276 ± 18^{a}	1.4 ± 0.2^{a}
	60 days of administration		
0	6.7 ± 0.3^{a}	381 ± 20^{a}	1.9 ± 0.4^{a}
50	7.2 ± 0.4^{a}	$448 \pm 17^{a,b}$	2.0 ± 0.4^{a}
100	$7.6 \pm 0.3^{a,b}$	$435 \pm 19^{a,b}$	1.6 ± 0.2^{a}
200	8.4 ± 0.3^{b}	472 ± 30^{b}	1.7 ± 0.3^{a}
	90 days of administration		
0	6.8 ± 0.6^{a}	541 ± 33^{a}	0.9 ± 0.2^{a}
50	7.0 ± 0.6^{a}	527 ± 30^{b}	1.0 ± 0.2^{a}
100	6.6 ± 0.4^{a}	555 ± 20^{a}	1.3 ± 0.3^{a}
200	7.2 ± 0.3^{a}	548 ± 36^{a}	0.8 ± 0.2^{a}
	120 days of administration		
0	6.9 ± 0.2^{a}	354 ± 10^{a}	1.4 ± 0.2^{a}
50	8.1 ± 0.3^{b}	391 ± 18^{b}	1.8 ± 0.4^{b}
100	$7.3 \pm 0.6^{a,b}$	$411 \pm 9^{b,c}$	1.6 ± 0.4^{a}
200	$7.5 \pm 0.1^{a,b}$	440 ± 8^{c}	1.0 ± 0.2^{a}

a-c Values with same superscript are not significantly different ($P>0.05$).

Subacute to Longer-term Exposures

A number of reports have appeared in the literature that describe effects following subacute to longer-term exposures of experimental animals to the ortho- and para- isomers of dichlorobenzene. (See Table V-6 for a summary of these studies).

Ortho-dichlorobenzene

Hollingsworth, et al. (1958) gave rats a series of 138 doses of o-DCB over a period of 192 days (18.8, 188 or 376 mg/kg/day, five days a week) by intubation in oil. No adverse effects were detected at the lowest dose. With the intermediate dose, a slight increase in the weight of the liver and kidneys was noted. At the highest dose, there was moderate increase in the weight of the spleen and cloudy swelling of the liver.

Hollingsworth, et al. (1958) also measured the effects following inhalation exposures of o-DCB for rats, guinea pigs, mice, rabbits and monkeys. The animals were subjected to a range of vapor concentrations, seven hours a day, five days a week, for six to seven months. No adverse effects were observed in rats, guinea pigs or mice exposed to 49 ppm, or in rats, guinea pigs, rabbits and monkeys exposed to 93 ppm.

Oral administration of o-DCB to white rats for nine months was conducted at doses of 0.001, 0.01 or 0.1 mg/kg/day (Varshavskaya, 1968). Effects were observed at the two higher doses similar to those described by this author for chlorobenzene. She reported an inhibition of mitosis in the bone marrow, as well as neutropenia and abnormal conditioned reflexes. In this report,

Table V-6

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed CNS	Blood	Liver	Kidney	Lung	Bone Marrow	Remarks	Reference
o-DCB	Rat	18.8,188 or 376 mg/kg/day	5 days/week, 138 doses 192 days	Oral			+	+			No adverse effects at 18.8 mg/kg. Slight increase in kidney and liver weight at 188 mg/kg. Moderate increase in liver weight and cloudy swelling, decrease in spleen weight at 376 mg/kg.	Hollingsworth, et al. (1958)
	Rat	0.001,0.01 or 0.1 mg/kg day	9 mos.	Oral	+	+	+			+	No adverse effects at 0.001 mg/kg Dose-dependant changes in conditioned reflexes, depression of hematopoietic system at 0.01 and 0.1 mg/kg.	Varshavskaya (1968)
	Rat(20) Guinea pig (8) Mouse (108)	49 ppm	7 hrs/day; 5 days/week; 6-7 months	Inhalation							No adverse effects observed	Hollingsworth, et al. (1958)
	Rat(20) Guinea pig (8) Rabbit (9) Monkey (28)	93 ppm	7 hrs/day; 5 days/week; 6-7 months	Inhalation							No adverse effects observed	"

( )= Number of animals tested

Tabel V-6 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed CNS	Blood	Liver	Kidney	Lung	Bone Marrow	Remarks	Reference
p-DCB	Guinea pig	125 or 250 mg/day as 50% soln or 125 mg/kg in olive oil.	10-11 days	Intra-muscular (I.M)		+	+				Intense steatosis of liver; weight loss; decreased hepatic glycogen	Frada and Cali, (1958)
	Guinea pig	125 mg/kg	20 days	I.M.	+		+				Weight loss; serum transaminase increased	Totaro, (1961)
	Guinea pig	125 (m)g/ (kg)	20 days	I.M.	+	+					11.4% weight loss; serum transaminase increased	Totaro and Licari (1964)
	Guinea pig	125 (m) g/ (kg)	21 days	I.M.			+				Increase in reaction and clotting formation time	Coppola, et al. (1963)
	Rat	10, 100 or 500 mg/kg as 10% soln.	5 days/week; 20 doses	Oral			+	+			No adverse effects observed at 10 or 100 mg/kg; cloudy swelling and centrolobular necrosis of liver; cloudy swelling of renal tubular epithelium	Hollingsworth, et al. (1956)

Table V-6 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed						Remarks	Reference
					CNS	Blood	Liver	Kidney	Lung	Bone Marrow		
	Pekin duck	0.5% in Diet	35 days	Oral							Growth retardation; no cataracts; 3 deaths after 4 weeks on diet	Hollingsworth, et al. (1956)
	Rabbit	500 mg/kg day	5 days/week; 263 doses	Oral			+				Swelling and focal necrosis in liver	"
		1000 mg/kg day	5 days/week; 92 doses over 219 days	Oral	+	+	+				Weight loss; tremors and weakness; necrosis and cirrhosis in liver	"
	Rat	18.8,188 or 376 mg/kg/day	5 days/week; 138 doses in 192 days	Oral			+		+		No adverse effects observed at 18.8 mg/kg. Increased liver and kidney weights at 188 and 376 mg/kg. Decreased spleen weight; liver necrosis and cirrhosis at 376 mg/kg.	"

Table V-6 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species (No.males(M)) (No.females(F))	Dose	Duration	Route	Effects Observed CNS	Blood	Liver	Kidney	Lung	Bone Marrow	Remarks	Reference
	Rat (9M,9F)	100 mg/l (16,000 ppm)	20 min/day; 5-9 days	Inhalation	+	+	+	+	+		Granulocytopenia in 8/18; tendency toward same in 3/18	Zupko and Edwards (1949)
	Guinea pig (9M)				+	+	+	+	+		Granulocytopenia in 5/9; tendency toward same 2/9; weight loss in 6/9	"
	Rabbit (18M)		30 min/day		+	+	+	+	+		Granulocytopenia in 11/18 Irritation of mucosa, weight loss in 14/18; Tremors rapid but labored breathing, death 12/18.	
	Rat (10M)	96 ppm,	7 hrs/day; 5 days/week; 16 days	Inhalation							No adverse effects observed	Hollingsworth et al.(1956)
	(10)	158 ppm	"				+	+			Cloudy swelling or granular degeneration of liver	"
	(5)	173 ppm	"			+	+	+	+		Slight interstitial edema and congestion in lung; slight increase in liver and kidney weights.	"

Table V-6 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed CNS	Blood	Liver	Kidney	Lung	Bone Marrow	Remarks	Reference
	Rat (19M) (15F)	798 ppm	8 hrs/day; 5 days/week; 1-46 doses (M) 9-69 doses (F)		+		+	+ (F)			Tremors; weakness; cloudy swelling of liver (M&F) and kidney (F), Deaths: 2M, 2F	"
	(20M)	341 ppm	7 hrs/day; 5 days/week, 6 months				+	+			Slight increase in liver and kidney weights	"
	Guinea pig (5)	173 ppm	7 hrs/day; 5 days/week; 16 days		+	+	+ (F)	+ (M)			Slight decrease in spleen weight, slight edema and congestion in lungs	Hollingsworth et al.(1956)
	(16M) (7F)	798 ppm	8 hrs/day; 5 days/week 1-23 doses (M) 11-20 doses (F)		+		+				Deaths: 2M	"
	(8)	96 ppm	7 hours/day; 5 days/week 157-219 days								No adverse effects observed	"
	(8)	158 ppm	"				+ (F)				Increased liver weight (F); Body weight loss	"

Table V-6 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed						Remarks	Reference
					CNS	Blood	Liver	Kidney	Lung	Bone Marrow		
	Rat (8M) (8F)	341 ppm	7 hours/day; 5 days/week; 6 months		+		+				Cloudy swelling, fatty degeneration, focal necrosis, slight cirrhosis of liver	"
	Rabbit (1M) (1F)	173 ppm	7 hours/day; 5 days/week; 16 days						+		Slight edema and congestion of lungs	"
	(8M) (8F)	798 ppm	8 hours/day; 5 days/week; 1-62 exposures		+		+		+		Reversible non-specific eye changes	Hollingsworth et al. (1956)
	(1M) (1F)	158 ppm	7 hours/day; 5 days/week; 157-219 days								No adverse effects observed	"
	Mouse	96 ppm	7 hours/day; 5 days/week; 157-219 days								No adverse effects observed	"
	(10)	158 ppm	"								No adverse effects observed	"
	Monkey (18)	158 ppm	"								No adverse effects observed	"

she also measured tissue DPN, TPN, glucose-6-phosphate and alkaline phosphatase. At the 0.1 and 0.01 mg/kg doses, o-DCB caused an increase in acid phosphatase but a decrease in alkaline phosphatase. The 0.1 mg/kg dose of o-DCB caused a marked increase in the amount of 17-ketosteroids found in the urine. This increase may be to hyperplasia of the adrenal cortex, since an increase in the weight of the adrenals and decrease in the ascorbic acid concentration of the adrenals also were observed. The 0.001 mg/kg dose had no observable effects on any of the parameters studied.

Para-dichlorobenzene

Intramuscular injection of 125 or 250 mg p-DCB/kg into guinea pigs over a 10 or 20 day period resulted in effects typical of p-DCB toxicity (Frada and Cali, 1958; Totaro, 1961; Totaro and Licari, 1964; Coppola, et al., 1963). The observed changes included weight loss, liver changes and blood clotting time increases.

Oral doses of 10, 100 or 500 mg p-DCB/kg in oil, five days a week, for 20 doses produced marked cloudy swelling and necrosis in the central area of the liver lobules only with the highest dose (Hollingsworth, et al.,1956). No effects were observed at the other doses. Pekin ducks, fed 0.5% p-DCB in their diets for 35 days exhibited depression of body weight gain. Three animals died after the fourth week.

Oral doses of 188 or 376 mg p-DCB/kg in oil, five days a week, for 192 days (138 doses) in rats induced an increase in the weights of the liver and kidneys (Hollingsworth, et al., 1956). At 376 mg/kg, decreased splenic weight, slight cirrhosis and focal necrosis of the liver also were observed. There also was cloudy swelling of the renal tubular epithelium. No adverse effects were seen with the 18.8 mg kg dose. Rabbits receiving 500 mg/kg oral doses, 5 days a week, for a total of 263 doses, showed swelling and focal necrosis of the liver. Other rabbits receiving a total of 92 oral doses at 1000 mg/kg/day over a 219-day period exhibited necrosis and cirrhosis of the liver. CNS effects were evident as well, as both tremors and weakness were noted.

Hollingsworth, et al. (1956) conducted a series of inhalation studies with p-DCB, at various levels and durations of exposure, in rats, guinea pigs, monkeys, rabbits and mice. The levels used used were 96, 158, 173, 341 and 798 ppm (0.58, 0.95, 1.04, 2.05 and 4.8 mg/l, respectively). Rats exposed to 96 ppm seven hours a day, five days a week, for 16 days showed no abnormalities. A 157-219 day exposure at this level in guinea pigs and mice caused no adverse reactions. Inhalation levels of 158 and 173 ppm over 7 hours/day, 5 days/week caused cloudy swelling or granular degeneration of the liver, slight increase in the weight of the liver and kidneys and some interstital edema and congestion in the lungs of rats and guinea pigs. Monkeys and mice showed no adverse effects at these levels. Rats exposed to 341 ppm for six months showed evidence of a slight increase in the weight of the liver and kidneys. Guinea pigs exposed to 173 ppm for 16 days showed a slight decrease in splenic weight and some lung edema and congestion. Guinea pigs exposed at 341 ppm for six months exhibited hepatic changes including slight cirrhosis with focal necrosis, cloudy swelling and fatty degeneration. Rabbits, exposed to an atmosphere of 158 ppm for

157-219 days, were unaffected. Inhalation of 173 ppm for 16 days produced slight edema and lung congestion. Reversible, unspecified eye changes were apparent in rabbits subjected to 798 ppm for 1-62 exposures.

A number of investigators have suggested that a relationship exists between the induction of -aminolevulinic acid synthetase and the induction of drug or xenobiotic metabolizing systems by compounds which are known to induce liver porphyria (Rimington and Ziegler, 1963; Poland, et al., 1971; Ariyoshi, et al., 1975a,b).

Poland et al. (1971) treated young female Sherman rats by gavage with daily doses of m-DCB in peanut oil. Continuous daily dosing with 800 mg/kg/day for nine days resulted in the pattern of urinary coproporphyrin excretion shown in Figure V-1. ALA synthetase was measured in animals dosed daily with 800 mg/kg/day for 1, 3 or 5 days. Enzyme activity measured 24 hours after the last dose showed a steep rise after Day 1, with lesser increases seen after Days 3 and 5 (Table V-7). Increases in liver size were not sufficient to account for the changes observed. No histological evidence of liver damage was noted in these animals. The nature of the response suggests that an adaption or tolerance to the compound is developing, perhaps because the animal may be metabolizing the substance more rapidly. This possibility was tested by observing the effects of the same dosing regimen as described above on hexobarbital sleeping time (150 mg/kg i.p. followed by recording of the time required for the return of the righting reflex). Sleeping times were significantly shortened after one 800 mg/kg dose of m-DCB (from 180 minutes to less than 120 minutes); after

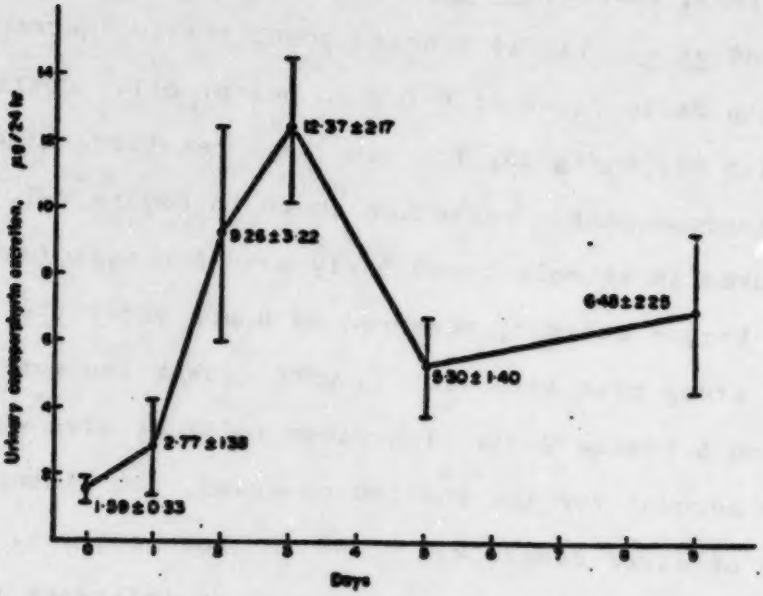


Fig. V-1. Effect of m-DCB on urinary coproporphyrin excretion. Four female rats (90-120 g) were treated daily with 800 mg/kg m-DCB. The 24-hr urinary excretion of coproporhyrin (mean + S.D.) is plotted versus time. The first dose was given on day 0, and the urinary coproporphyrin value for each day represents a urinary collection for the previous 24 hr. Urinary coproporphyrin excretion was significantly lower on days 5 and 9 than on day 3.

Source: Poland, *et al*. (1971).

Table V-7

Effect of m-Dichlorobenzene on ALA Synthetase Activity*

Treatment	ALA synthetase (mmoles/g/hr) Day 1	 Day 3	 Day 5
Control	13.3 ± 4.4 (7)	18.8 ± 4.6 (6)	16.1 ± 4.8 (7)
m-DCB	52.9 ± 15.3 (7)	40.5 ± 6.8 (7)	30.5 ± 6.2 (8)

* Female rats were treated orally for 1,3 or 5 days with m-DCB (800 mg/kg) or peanut oil and sacrificed 24 hr after the last dose. All values represent the means ± S.D. The number of animals in each group is given in parentheses.

ALA synthetase activity is lower on day 5 in m-DCB-treated animals than on day 1 ($P<0.01$) or day 3 ($P<0.02$).

Source: Poland, et al. (1971)

three daily doses, sleeping times decreased to 20% of control values (30 minutes vs. 180 minutes;$p<0.001$). Acceleration of the rate of metabolism of bishydroxycoumarin (BHC) also occurred after treatment with five consecutive daily doses of 800 mg/kg m-DCB. Twenty-four hours after the last dose of m-DCB or vehicle (peanut oil) was administered, the rats were injected i.p. with 20 mg/kg BHC in saline. Five hours later, blood was drawn via cardiac puncture under ether anesthesia. After the five days of dosing, the serum BHC levels measured 82 ± 24 ug/ml, as compared with control levels of 133 ± 10 ug/ml ($P<0.025$).

Poland et al. (1971) conducted further experiments designed to test the hypothesis that m-DCB stimulates its own metabolism. Liver and serum levels of m-DCB and 2,4-dichlorophenol (DCP), its major metabolite, were determined in rats after dosing daily for up to five days, as described above. The serum m-DCB concentration was higher on day 3 (8.89 ± 1.61 ug/ml) than on day 1 (3.25 ± 1.31 ug/ml), but was significantly lower on day 5 (5.91 ± 2.02 ug/ml) than on day 3 ($P<0.02$). The hepatic concentration rose steeply from day 1 (13.01 ± 6.92 ug/g tissue) to day 3 (44.17 ± 6.31 ug/g). However, the difference between the concentration at day 3 and 5 (32.1 ± 16.18 ug/g) was not quite statistically significant ($0.10>P>0.05$). 2,4-DCP could not be detected in the serum or liver of any of the animals. In rats pretreated for four days with 40 mg/kg phenobarbital i.p., a potent inducer of drug metabolism, before receiving a single 800 mg/kg dose of m-DCB, slightly lower concentrations of m-DCB in liver (8.49 ± 2.37 ug/g) and serum (2.24 ± 0.49 ug/g) were observed as compared with those in rats receiving only a single dose of m-DCB. The differences were not significantly different, however. The data from this series of experiments add support to the hypothesis that m-DCB does, in fact, stimulate its own metabolism.

Ariyoshi et al. (1975a,b) studied changes in certain liver constituents, cytochrome contents, activities of some drug-metabolizing enzymes and Δ-ALA synthetase in rats treated with each of the three isomers of DCB. Animals received oral doses of 250 mg/kg/day for up to three days. Activities of aminopyrine demethylase and aniline hydroxylase were enhanced markedly by m-DCB, but cytochrome P-450 content was not altered significantly after any of the three isomers. Δ-ALA synthetase activity was increased significantly by treatment with o-, m- and p-DCB (63%, 32% and 42%, respectively). However, significant parallel changes in the cytochrome P-450 content did not occur. All isomers increased microsomal protein content of liver preparations. Microsomal inorganic phosphorous content also was increased by 36% after treatment with m-DCB.

Carlson and Tardiff (1976) studied the effects of several halogenated benzenes, including 1,4-dichlorobenzene, on adult male rats. The compounds were given orally for 14 days at low doses of 10 to 40 mg/kg body weight. 1,4-DCB decreased hexobarbital sleeping time 24 hours after cessation of a 14-day treatment period. In addition, as can be seen in Table V-8, administration of 1,4-DCB for 90 days resulted in increases in glucuronyl transferase levels, EPN detoxication, benzpyrene hydroxylation and azoreductase levels. The increases were still apparent 30 days after cessation of exposure.

Table V-8

Effect of 1,4-Dichlorobenzene Administered Orally for 14 Days on Various Parameters of Xenobiotic Metabolism

(Carlson and Tardiff, 1976)
(Modified from Ware and West, 1977)

Compound	Dose (mg/kg/day)	Cytochrome c reductase (nmol cytochrome c reduced/min mg protein)*	Cytochrome p-450 (E/mg protein x 10)	Glucuronyl-transferase (nmol/min/mg protein)	EPN$^+$ detoxication (pg p-nitrophenol/ 50 mg/30 min)	Benzpyrene hydroxylase (nmol/mg protein/min	Azoreductase (ng/min/mg protein)
1,4-Dichloro-benzene	0	156 ± 9^b	178 ± 20^b	5.9 ± 0.6^b	6.7 ± 0.6^b	2.36 ± 0.49^b	63.9 ± 2.8^b
	10	151 ± 6^b	174 ± 15^b	7.8 ± 0.6^{b,c}	7.2 ± 0.6^{b,c}	1.75 ± 0.13^b	67.5 ± 2
	20	165 ± 8^b	167 ± 11^b	11.3 ± 1.0^d	9.5 ± 1.1^{c,d}	3.53 ± 0.68^c	71.4 ± 4.3
	40	176 ± 10	193 ± 13^b	9.6 ± 0.6^{c,d}	9.0 ± 0.5^d	2.15 ± 0.34^b	79.2 ± 6.6

* Value is mean ± S.E. for group of six rats except for benzpyrene hydroxylase group receiving 40 mg/kg of 1,2,4-

+ EPN = o-Ethyl o-p-nitrophenylphenylphosphonothioate

$^{b-d}$ Values which have been assigned the same superscript do not differ significantly ($p>0.05$)

Multiple Chemical Exposures: Actions of Combinations

Since many compounds, whether they are agricultural and industrial chemicals or drugs, are handled similarly by the cytochrome P-450 system, there are possibilities for additive, synergistic as well as antagonistic actions.

There are a few examples of synergistic effects of halogenated benzenes on a target organism found in the literature. Hinze, et al. (1970) showed that the antifungal activity of halogenated benzenes was synergistic with organo-tin compounds. The mechanism of the toxicity was not discussed.

Several halogenated benzenes, including p-DCB, have been studied for their effects on the metabolism of malathion, malaoxon and paraoxon and on the toxicity and lethality of these organophosphorous compounds as well as parathion (Townsend and Carlson, 1981). In studies to determined the lethality of these compounds, p-DCB, in doses of 1.0 mmol/kg (250 mg/kg body weight) in corn oil, was administered orally to male mice daily for 7 days. Twenty-four hours after the last dose, the mice were given graded doses of malathion, parathion and paraoxon by gavage in corn oil. Another twenty-four hours later, the number of dead animals was counted and the LD_{50}s calculated. Little effect was noted on the LD_{50} of malathion (1404 mg/kg after p-DCB vs. 1370 mg/kg in the controls). On the other hand, p-DCB did increase the LD_{50} for parathion (18.1 mg/kg after p-DCB vs. 12.1 mg/kg in controls) and for paraoxon (15 mg/kg after p-DCB vs. 11.5 mg/kg in controls).

The possible mechanism of protection against lethality to malathion and malaoxon also was investigated by Townsend and Carlson.

They hypothesized that the rate of metabolism of these organophosphate pesticides was increased. This hypothesis was tested by measuring the ability of the plasma and liver of the mice to hydrolyze malathion and malaoxon. After dosing with 250 mg/kg p-DCB for 7 days, the liver and plasma of the treated mice were analyzed for carboxylesterase activity with each chemical as the substrate. After treatment with p-DCB, the amount of malathion hydrolyzed in the liver was increased significantly to 7.43 ± 0.78 umol/g tissue/min from 3.81 ± 0.39 umol/g tissue/min in controls ($p<0.05$). The change in plasma was not statistically different (4.81 ± 0.45 in controls vs. 6.07 ± 0.47 after p-DCB). Pretreatment with p-DCB did not affect the rate of malaoxon hydrolysis either in liver or plasma.

The same authors also investigated the effect of halobenzenes on the metabolism of parathion and paraoxon, specifically via dearylation, a reaction carried out by an esterase and a mixed function oxidase. Again, mice were pretreated with p-DCB as described above. Plasma esterase, hepatic soluble esterase, hepatic microsomal esterase and microsomal mixed function oxidase were studied. p-DCB had no effect on plasma esterase, hepatic soluble esterase or microsomal esterase levels. However, p-DCB did cause a significant increase in the activity of microsomal mixed function oxidase (1.09 ± 0.41 mmols p-nitrophenol formed/min/mg protein in controls vs. 4.13 mmols in the p-DCB-pretreated). The results of both sets of experiments suggest that, in fact, the metabolism of the parent compounds, in particular, is increased in rate to some degree.

Teratogenicity

Several teratogenicity studies have been conducted with two of the dichlorobenzene isomers (ortho- and para-).

Hodge, et al. (1977;summarized in Loeser and Litchfield, 1983) conducted a teratogenicity study of p-DCB in rats. Groups of pregnant rats (32 animals per group) were exposed to atmospheric concentrations of 0, 75, 200 or 500 ppm p-DCB 6 hours/day on days 6-15 of pregnancy. Data were collected only from the first 20-24 animals in each group proven to be pregnant at the time of sacrifice (day 21). Nine animals littered spontaneously on day 21 and thus were not included in the study results (two at 75 ppm, two at 200 ppm and five at 500 ppm).

Maternal weight gain was monitored over the 21 days of pregnancy. Exposure to p-DCB did not alter the rate of weight gain in any group when compared with controls.

On day 21, the animals were sacrificed. The intact uteri were examined for numbers of fetuses and resorptions. Corpora lutea were counted. Upon dissection of the uteri and removal of the fetuses, the resorptions which had occurred were classified as early or late. Resorptions are designated as late when fetal tissues are distinguishable. When abnormal fetuses were noted, maternal heart, liver, lung, uterus, ovary, kidney and adrenal were preserved for histological examination. Liver and lung from at least ten animals/group also were fixed for examination.

Each fetus was examined for viability, sex, weight and presence of abnormalities. Half of each litter was eviscerated, examined for abdominal abnormalities and stained with Alizarin Red for subsequent skeletal examination for abnormalities and degree of ossification.

Upon gross examination, uteri from three females exposed to 75 ppm contained excessive amounts of blood. In one case, this appeared to be associated with a dead fetus. One female exposed to 200 ppm had inflated lungs. Upon histological examination, no lesions were observed which were attributable to exposure to p-DCB.

Exposure to p-DCB did not induce adverse effects on numbers of implantations, viable fetuses, resorptions, corpora lutea or on mean fetal weights, mean litter weights or on implantation efficiency (number of implantations/number of corpora lutea). Sex ratios (male/female) were within normal limits. There was no increase in the number of runts.

Three gross fetal abnormalities were noted, one in each experimental group. From the 75 ppm group, there was one fetus with gastroschisis and malrotation of the left hindlimb. In the 200 ppm group, there was a single fetus with gastroschisis and malrotation of the right hindlimb, and in the 500 ppm group, there was one fetus with agnathia and cleft palate.

Upon examination for skeletal abnormalities, no evidence was found that maternal exposure to p-DCB resulted in delayed ossification of fetal bones or an increased incidence of minor abnormalities. Occasionally, 14 ribs were noted, but, in almost all cases, these were vestigial (see Table V-9).

The results of this study by Hodge, et al. indicate that maternal exposure to atmospheric levels of p-DCB up to 500 ppm on days 6-15 of pregnancy does not result in any embryotoxic, fetotoxic

Table V-9

Occurrence of Vestigial Ribs in Rat Fetuses Exposed to p-Dichlorobenzene in utero

(Hodge, et al., 1977)

Observation	Treatment Group							
	0 ppm		75 ppm		200 ppm		500 ppm	
	No.	%	No.	%	No.	%	No.	%
14 left ribs (1st lumbar)	11	8.9	8	7.3	12	11.8	7	6
14 right ribs (1st lumbar)	7	5.7	2	1.8	6	5.9	1	0.9
14 bilaterally (1st lumbar)	23	18.7	6	5.5	21	20.6	15	12.8
14 left (7th cervical)	0	0	1	0.9	0	0	0	0
Total # fetuses with 14 ribs	41	33.3	17	15.6	39	38.2	23	19.7
12 left	0	0	0	0	0	0	1	0.9
"kinky"	0	0	1	1.9	0	0	1	0.9

or teratogenic effects in the offspring of rats.

Dow Chemical Company has completed studies in which pregnant rats and rabbits were exposed to o- and p-DCB via inhalation (Hayes, et al., 1985). Results of a range-finding study to determine the maximum tolerated dose of o-DCB for the full study, as well as the final protocol for that study were submitted to EPA earlier (John, et al., 1980, 1981). In the probe study, groups of 10 pregnant rats and seven pregnant rabbits were exposed to nominal o-DCB concentrations of 0, 200, 400, or 500 ppm (0, 1200, 2400 or 3000 mg/m^3). The animals were exposed six hours/day on days 6-15 (rats) or days 6-18 (rabbits) of gestation.

Severe maternal toxicity, as evidenced by significant decreases in body weight, body weight gain and food consumption, increases in relative liver and kidney weights and signs of systemic toxicity at gross necropsy, was observed in pregnant rats exposed to 500 ppm of o-DCB. Embryolethality, secondary to maternal toxicity, also was observed among rats in the 500 ppm exposure group. Increased relative liver and kidney weights and decreased food consumption were observed among pregnant rats exposed to 400 ppm of o-DCB. Exposure to 200 ppm of o-DCB did not produce any sign of toxicity among maternal animals. No statistically significant effects on reproductive parameters were observed among rats at any exposure level.

In the rabbits, slight maternal toxicity was observed among pregnant animals exposed to 500 ppm of o-DCB. Non-significant decreases in maternal body weight gain, absolute and relative liver weight and liver:body weight ratio were observed among rabbits exposed to 500 ppm. Gross observation at necropsy revealed hepatic changes indicative of mild toxicity among pregnant rabbits exposed to 500 ppm. No adverse effects were observed among rabbits exposed to 200 or 400 ppm of o-DCB, and no significant effects on reproductive parameters were observed among rabbits at any exposure level.

Based on the results of the probe study, as summarized above, exposure concentrations of 0, 100, 200 and 400 ppm o-DCB were chosen as the test concentrations for a comprehensive teratology study in rats and rabbits.

As reported in Hayes, et al. (1985), groups of 30 or 32 bred rats or 28 or 30 inseminated rabbits were exposed to p-DCB at the levels noted above. The animals were exposed 6 hours/day, on days 6-15 (rats) or 6-18 (rabbits) of gestation. Controls were exposed to filtered air in individual chambers for 6 hours/day over the same time periods.

In the rats, maternal toxicity was evident in all o-DCB-exposed groups. Mean body weight gains were significantly lower then controls for the time interval of gestation days 6-10 (18-27% decrease). In addition, a significant increase in both absolute and relative liver weights was noted in rats exposed to 400 ppm, and in relative liver weight of animals exposed at 100 ppm.

No embryotoxicity or fetotoxicity was observed in litters from any group, as measured by litter size, resorption rate or fetal body measurements. No treatment-related teratogenic changes were identified. Among the controls, 4 fetuses in 1 litter were missing 1 pair of ribs and 1 thoracic vertebra; 2 of these 4 fetuses had cervical ribs as well as did a single fetus from a second litter. In the 100 ppm group, 3 fetuses in 3 litters had cervical ribs, as did 1 fetus in the 200 ppm group.

Among fetuses in the 400 ppm group, 5 fetuses from 3 litters had major malformations consisting of single cases of coarcted and retroesophageal aortic arch, unilateral testicular agenesis, polydactyly, cervical ribs and microopthalmia. Skeletal abnormalities characterized by significant occurrences of spurs on the first lumber vertebra and delayed ossification of the sternebrae were observed in fetuses from the 200 ppm group. These effects were not significantly increased in number in the 400 ppm group, although the occurrence of delayed ossification of cervical vertebral centra was greater than in the control group.

In the rabbits, dams in all groups showed a significant weight loss during the first three days of exposure. The body weight gain of animals in the lowest exposure group was significantly lower than controls over the total study duration(days 6-28 of gestation). However, neither liver nor kidney weights were affected at any dose.

As with the rats, there was no evidence of fetotoxicity or embryotoxicity in the rabbits. The sex ratio was significantly altered in the 200 ppm group when compared with controls (male:female = 61:39 at 200 ppm and 51:49 at 0 ppm).

The incidences of major malformations was not increased significantly for any of the exposed groups when compared with controls. One fetus from the control and 400 ppm groups had anencephaly, aprosopia (missing facial features) and forelimb flexure. These two also exhibited agnathia (missing jaw) and epitheliogenesis imperfecta (focal lack of formation of skin and adnexa). Also in the control group, there was another fetus with forelimb flexure, one with calloused ribs, and a third with multiple malformations. In the 100 ppm group, a single fetus had a dilated renal pelvis, single fetuses from two litters showed forked ribs, and one fetus from another litter had multiple malformations. In the 200 ppm group, one fetus had coarctation of the aortic arch and another from a second litter, a missing vertebra.

Additional malformations in the 400 ppm group included one fetus with forelimb flexure and rotated hind limb, and a second with a rotated hind limb. One fetus from another litter had multiple malformations and a second fetus from the same litter had a missing vertebra and fused ribs. Also, there were single cases of fused ribs, missing vertebrae and hemivertebra.

Dow Chemical Company also submitted to EPA the results of a dose range-finding study of p-DCB exposure to pregnant rabbits (Hayes, et al., 1982). Pregnant females were exposed to 0, 300, 600 or 1000 ppm (0, 1800, 3600 or 6000 mg/m^3). Each was exposed for 6 hrs/day on days 6-18 of gestation.

No maternal deaths occurred during the study and no significant changes in gross appearance or demeanor were observed among p-DCB-exposed rabbits. Evidence of slight maternal toxicity was observed among pregnant rabbits exposed to 1000 ppm of DCB. In this group, a decrease in body weight gain and slight decreases in absolute liver weight and liver:body weight ratio were observed. In addition, histopathologic examination of livers revealed decreased hepatocellular vacuolization suggestive of decreased glycogen deposition in the

1000 ppm group.

No significant effects on the incidence of implantations undergoing resorption were observed in any of the exposed groups when compared to controls, indicating that p-DCB was not embryolethal to rabbits at exposure concentrations up to 1,000 ppm.

Based on the results of this probe study, where evidence of slight maternal toxicity was observed in the 1000 ppm group, exposure levels of 100, 300 and 800 ppm of p-DCB were selected for a comprehensive teratology study in rabbits. Hayes, et al. (1985) reported the results of this study. The experimental protocol was identical to that described above for o-DCB. Some maternal toxicity, manifested as a slightly reduced body weight gain, was observed in the 800 ppm group. One pregnant rabbit in the 300 ppm group died on day 10, of causes unrelated to treatment.

A statistically significant increase in the incidence of implantations undergoing resorption was observed in the 300 ppm group (63% vs. 29% in the controls). This effect was not seen in either of the other two treatment groups. The investigators claimed that the 63% was within the range historically observed in that laboratory (0-70%), and did not consider the effect to be treatment-related since there was no dose response. Other parameters reflecting feto- or embryotoxicity were not altered significantly when compared with controls.

Major malformations did not occur at significantly increased rates in the treated groups when compared with controls. In the untreated group, one fetus had spina bifida and fused vertebrae. Single occurrences among the controls also included dilatation of aortic arch with precocious closure of the ductus arteriosus, coarctation of the aortic arch, forked and calloused ribs and hemivertebra. Two fetuses in 1 litter exhibited dilated renal pelvis.

At the lowest exposure dose (100 ppm), there were 2 fetuses in 1 litter with forelimb flexure. In 2 fetuses from different litters, there were calloused. In addition, there were single occurrences of hypoplastic thoracic vertebrae, fused ribs and fused vertebrae. At the intermediate dose (300 ppm), single fetuses in three litters had fused ribs, forelimb flexure and calloused ribs. At the highest dose, 1 fetuses in 1 litter was anencephalic and possessed forelimb flexure and umbilical hernia. Two other fetuses in this litter exhibited forelimb flexure and shortened long bones. Three fetuses from a single litter exhibited malformations including dilated cerebral ventricles, umbilical hernia, dilated renal pelvis and fused ribs and vertebrae. In addition, single occurrences of coarctation of the aortic arch, calloused ribs, extra rib fused to the tenth rib and a right subclavian artery originating off the pulmonary trunk were observed.

A statistically significant increase in the incidence of a retroesophageal right subclavian artery was observed in fetuses from dams treated at 800 ppm (five litters vs. one control litter). The investigators did not consider this effect to be indicative of a teratogenic response, stating that this variation had previously been observed in 2% of the litters from control rabbits in that laboratory.

Mutagenicity

Effects on Plants

Abnormal mitotic division of the onion, Allium cepa, after treatment with several halogenated benzenes has been described by Ostergren and Levan (1943). Ortho-DCB produced full c-mitosis abnormalities at 300 x 10^{-6} mol concentration with partial disturbances of mitotic division at 100 x 10^{-6} mol. Meta-DCB produced full c-mitosis abnormalities at 300 x 10^{-6} mol concentration, but had no effect at 100 x 10^{-6} mol.

Available studies with p-DCB are summarized in Table V-10.

Effects on Microorganisms

Anderson et al. (1972) evaluated 110 herbicides for their ability to produce point mutations in a number of different microbial systems. When tested in culture of eight different strains of histidine-requiring mutants of Salmonella typhimurium, o-DCB (dose not stated) gave a negative response, i.e., it was not mutagenic. No liver homogenates containing the metabolic activating enzymes were added in order to study the effect of conversion of the test compounds to active intermediates.

Prasad and Pramer (1968) and Prasad (1970) investigated the mutagenic effects of the three dichlorobenzene isomers. The chemicals were evaluated for frequency of back mutation of the methionine-requiring ($meth_3$) locus in the fungus Aspergillus nidulans. The mutagenicity of the dichlorobenzenes increased in the following order: o-DCB-5 revertants/10^6 spores, m-DCB-9 revertants/10^6 spores and p-DCB-11 revertants/10^6 spores.

Table V-10

Effects of p-DCB on Mitotic Division of Plants

(Modified after Ware and West, 1977)

Organism	Treatment	Fragments	Persistence Of Fragments	Remarks	Reference
Allium	0.05, 0.1, 0.25, 0.5, 1.5 g/dish for five days to seeds in Petri dish			No signs of mitosis at three highest doses	Carey and McDonough, 1943
	same doses to four-day old seedlings for four hours			Polyploidy in root cells at 0.5 and 1.5 g Abnormal chromosome numbers; lagging chromosomes and dumb bell shaped nuclei also occasionally seen	
Six species of monocotyledon angiosperms-root tips	4 1/2 hour soak in sat. soln.	+	+	Frequency of fragments at metaphase tended to decrease. Frequency high-24, 48 hrs. and decreased at 72, 96 hrs. 2 plants still highly fragmented at 96 hrs. 1 complete recovery.	Sharma and Battacharya, 1956

+ = Effect observed

− = Effect not observed

Table V-10 (Continued)

Organism	Treatment	Fragments	Persistence Of Fragments	Remarks	Reference
Nine species of dicotyledon angiosperms-root tips	1-4 hours depending on plant	+	+	2 plants recovered before 96 hrs. 1 plant died after 48 hrs. 3 plants very highly fragmented at 96 hours	Sharma and Bhattacharya, 1956
Nothoscordum fragrans Kunth					
Root tips	3 1/2 hrs. soak in sat. soln.	+	not indicated	Erosion and fragmentation of chromosomes both at metaphase and anaphase	Sharma and Sarkar, 1957
Root tips	6 hrs. sat.	++	not indicated	Erosion and stickiness increase in fragments from 3 1/2 hour soak.	Sharma and Sarkar, 1957
Pollen	6 hours	-	-	No fragmentation, some diplochromosomes. Indication of slight disturbance in spindle mechanism and failure in separation.	Sharma and Sarkar, 1957
(Flower Buds)	(3 1/2 hrs. sat. soln.)	(-)	(-)	(Meiotic division - only stickiness of chromosomes noted.)	Sharma and Sarkar, 1957
	(6 hrs. sat. sol.)	(-)	(-)	(Lagging, non-disjunction and stickiness of chromosomes. No fragments.)	Sharma and Sarkar, 1957

Table V-10 (Continued)

Organism	Treatment	Fragments	Persistence Of Fragments	Remarks	Reference
Three species of *Vicieae* root tips	4-6 hours in soln.	+	not indicated	c-Mitosis abnormalities. Breaks associated with heterochromatic chromosome regions	Srivastava, 1966
Fenugreek seeds	4-24 hours soak in sat. soln.	-	-	No change in morphology or cytology of seedlings	Gupta, 1972
Zea Mays L. (corn)	3 hour soak of 0.5 cm long seedlings in saturated aqueous solution, then growth allowed for 7 more days			Accelerated root growth, cell division; polyploidy, formation of chromosomal bridges and laggards at anaphase. Polarity of cell changed to an angle of 90° from normal.	Sharma and Agarwal, 1980
Lens esculenta micro sperma	4-48 hour soak of root tips in saturated aqueous solution 4-48 hour exposure of germinating seeds to vapors of 25, 50, 100, 250, 500, 800-1,000 mg of p-DCB crystals 4-48 hour soak of germinating root tips in saturated aqueous solution			Germiability and growth inversely proportional to level and duration of exposure. Mitotic and chromosomal anomalies observed: chromosome contraction and condensation, fragmentation, bridges, tetraploidy, binucleate cells	Sarbhoy, 1980.

Anderson (1976) reported on a study with p-DCB designed to estimate the mutagenic potential of this substance in the *Salmonella typhimurium* plate incorporation assay. Mutant strains TA 1535, TA 1538, TA 98 and TA 100 were exposed to varying concentrations of p-DCB dissolved in DMSO in two separate studies. In the first study, concentrations of 100, 500 and 2500 ug/plate were used. The experiment was run three times. In the second study, concentrations of 4 and 20 ug/plate were used in addition to the three concentrations used in the first study. This experiment was run five times, both with and without metabolic activation by S-9 mix from Arochlor-treated rats. In another series of experiments, the tester strains were exposed to atmospheric concentrations of p-DCB at levels of 94, 299 or 682 ppm, again with and without metabolic activation. This protocol was employed four separate times.

A greater than two-fold increase in the number of revertants is the criterion by which a compound is considered to be mutagenic in this assay system. After atmospheric exposure to p-DCB, no significant increases were noted in any tester strains, except in one of the four exposures to 682 ppm in TA 1535 with metabolic activation. This increase was not observed in three follow-up experiments. In the first series of experiments in which p-DCB was dissolved in DMSO at three concentrations, the number of revertants in TA 1535 increased nearly 10-fold at 500 ug/plate, with metabolic activation. This increase occurred in two of the three runs. This increase was not observed in the subsequent series of five runs. In spite of the few positive results, consideration of the data from all of the experiments together suggests that p-DCB is not mutagenic to tester strains used in this Salmonella typhimurium plate incorporation assay system, either when dissolved in DMSO or in the gaseous phase.

Simmon et al. (1979) examined all three dichlorobenzene isomers for mutagenic activity in both the standard Ames/Salmonella assay and the E. coli WP2 system. In the Ames assay, tester strains TA 1535, TA1537, TA1538, TA98 and TA100 were employed, with and without metabolic activation. In the first of two experiments, concentrations of 0.05-1.0 ul/plate (0.065-1.3 mg/plate) of o- or m-DCB were added to each Salmonella strain or E. coli culture. No reproducible dose-related increases in the number of revertants were observed in either system. In a second experiment, each compound was retested at lower levels, ranging from 0.0005 to 0.5 ul/plate. Again, no significant changes were noted. Para-DCB was tested in the same manner, but at higher dose levels: 50-1,000 ul/plate in the first experiment and 0.5-500 ul/plate in the second. Again, no increases in the number of revertants were observed.

Simmon, et al. (1979) also conducted tests for chromosomal aberrations in yeast. All three isomers were tested for their potential to induce mitotic gene conversion and reciprocal recombination in Saccharomyces cerevisiae C3, with and without metabolic activation. At doses ranging from 0.001-0.25%, o-DCB produced no effects. Toxicity was observed at 0.05%, with and without activation. Meta-DCB was tested at concentrations ranging from 0.005 to 0.1%

in two experiments. In both, but only after activation, enhancement of the recombination response was observed. Para-DCB, in concentrations ranging from 0.005-5% in the first three experiments, appeared to increase mitotic recombination, with a considerable variation in survival. In two later experiments, toxicity occurred but no increase in recombination was observed. The inconsistency of the results may be attributed to the relative insolubility of the compound.

The differential toxicity of each of the three isomers was evaluated in the DNA repair-proficient and repair-deficient strains of E. coli (W3110 polA$^+$/p3478 polA$^-$) and Bacillus subtilis (H17 rec$^+$/M45 rec$^-$) (Simmon, et al., 1979). In three of four experiments, 20 ug/plate o-DCB was more toxic to the repair-deficient E. coli (polA$^-$) than to the repair-proficient strain (polA$^+$). There was no apparent difference in toxicity to either strain of B. subtilis. At the same concentration (20 ul/plate), m-DCB was more toxic to the repair-deficient strain of E. coli than to the polA$^+$ strain in four of five experiments. Para-DCB, at concentrations of 1 or 5 mg/plate, had no effect on any of the four strains. This result was interpreted to mean that either the compound was truly nontoxic under these test conditions or that the substance was unable to diffuse away from the impregnated filter paper disc in the culture dish.

In summary, the results of these studies indicate that the dichlorobenzenes do possess mutagencity activity in certain of the test systems employed. None were positive in the Ames/Salmonella assay system or in the E. coli WP2 assay. However, m-DCB, both with and without metabolic activation, increased mitotic recombination in S. cerevisiae. The results with p-DCB were ambiguous. Both o- and m-DCB were shown to interact with and damage bacterial DNA in the E. coli W3110 polA$^+$/ p3478 polA$^-$ differential toxicity assay system.

Effects on Animals

There has been at least one in vitro study of the effect of dichlorobenzene (isomer not specified) on the number of mitoses in rat lung cell cultures (Guerin, et al., 1971). The dose of 5 ug did not produce any significantly different number of mitoses than in control cultures.

Cytogenetic studies have been conducted on rat bone marrow cells following inhalation exposure of rats to p-DCB (Anderson and Richardson, 1976). Three series of exposures were carried out: 1) one exposure at 299 or 682 ppm for two hours, 2) multiple exposures at 75 or 500 ppm, five hours/day for five days and 3) multiple exposures to 75 or 500 ppm, five hours/day, five days/week for three months. Benzene (at 10, 750 or 7500 ppm) was used as the positive control in the first experiment. Vinyl chloride (at 1500 ppm) was used as the positive control in the other two experiments. Negative controls breathed fresh air alone. In Experiment 1, three rats were used in each treatment group, and four were used in the negative control group. In the other two experiments, there were two rats in each treatment group and two rats in the negative control group. In Experiment 1, 50 cells from each animal were examined; in Experiments 2 and 3, 100 cells from each animal were examined.

Animals were sacrificed 22 hours after termination of exposure, one hour after an intraperitoneal dose of colchicine. Bone marrow cells from both femurs were stained with Giemsa and surveyed for chromosome or chromatid gaps, chromatid breaks, fragments or other complex abnormalities. In all three experiments, exposure to p-DCB failed to induce any statistically significant effects indicative of chromosomal damage when compared to the negative controls. The positive controls did produce damage at all levels employed in each experiment. Also, there was no evidence of a dose-response relationship to p-DCB exposure, whereas, there was with benzene in Experiment 1. Thus, under the conditions of these experiments, p-DCB does not appear to elicit chromosomal damage.

Para-dichlorobenzene also was tested in a dominant lethal study in the CD-1 mouse (Anderson and Hodge, 1976). Fertile males were exposed by inhalation to fresh air or to one of three levels of p-DCB, or by other routes of exposure to one of three substances known to produce dominant lethal effects in this assay system. Groups of males were subdivided into the following experimental groups. (Numbers of animals in each group are shown in parentheses).

Group 1: Air (negative control)-(35)

Group 2: 75 ppm p-DCB, 6 hr/day for 5 days (16)

Group 3: 225 ppm p-DCB, 6 hr/day for 5 days (16)

Group 4: 450 ppm p-DCB, 6 hr/day for 5 days (16)

Group 5: 200 mg cyclophosphamide/kg, once by i.p. injection on Day 5 (13)

Group 6: 150 mg ethyl methanesulfone/kg, orally once a day for 5 days (5)

Group 7: 2.5 mg nitrogen mustard/kg, once by i.p. injection on Day 5 (12)

The mating protocol consisted of placing two virgin females into a cage containing one fertile male. Five days later the females were removed to a separate cage. Two days later, the male was caged with two different virgin females. This process was repeated until the males had been mated at weekly intervals eight times. Females were sacrificed 13 days after the assumed date of fertilization, or 15-16 days after caging with the male. Uteri of these females were examined for live implants and early and late deaths. The data were analyzed statistically by a simple Chi-square and a week by week hierarchical analysis of variance.

Only one death occurred among males exposed to p-DCB. This was a male in the 75 ppm group during week 3. The investigators suggested that the death was unrelated to p-DCB exposure. Five males in the cyclophosamide positive control group died early, three during week 6 and one each in weeks 7 and 8.

Frequency of mating was affected in only two of the groups. At week 7, in the 77 ppm-dosed males, the frequency of mating was 100%, but significantly fewer males mated with both females ($P<0.05$).

The number and percentage of females becoming pregnant was decreased significantly at weeks 6 and 7 among those mated to males exposed to 75 ppm ($P<0.05$). The mean total of implants per pregnant female in each group also was determined. The ratio was reduced significantly in the 75 and 450 ppm groups at week 8 when compared with the negative control. All positive controls showed significant reductions at week 1 ($P<0.01$), cyclophosphamide and nitrogen mustard at week 2 ($P<0.01$) and ethyl methanesulfone at Week 8 ($P<0.05$) when compared with the negative controls.

Early fetal deaths were analysed in three ways. Groups exposed to 225 ppm p-DCB showed an increase at week 1 in the number of females with at least one early fetal death ($P<0.05$). All positive controls showed significant differences at weeks 1 and 2 ($P<0.001$ or 0.05). When comparing the mean number of early fetal deaths/pregnancy, there were no significant differences in any of the p-DCB-exposed groups. All positive control groups exhibited significant differences in week 1 and 2. Cyclophosphamide also exhibited an increase in Week 3 ($P<0.05$ or less).

Comparison of mean percentages of early deaths per total implants per pregnancy revealed significant differences in p-DCB-treated groups in week 6 in the 225 ppm group ($P<0.05$). Values for the 75 ppm group in week 1 were higher than the negative controls, but not significantly higher as determined by Dunnett's "t" test. No significant differences were seen in late fetal deaths for any groups.

Two of the three methods of analysis indicated significant differences between p-DCB-exposed groups and negative controls.

However, these changes were different at different times and they did not occur in a dose-related manner. Therefore, the authors suggested that these changes were not biologically significant. In addition, they concluded that p-DCB, at least at the exposure levels tested, does not cause dominant lethal mutations in germ cells of CD-1 mice.

Carcinogenicity

Few studies have appeared in the open, peer-reviewed literature which address the carcinogenic potential of the dichlorobenzenes.

Murphy and Sturm (1943) studied the effects of p-dichlorobenzene on induced resistance to a transplanted leukemia in the rat. Forty rats were immunized by intraperitoneal injection of either defibrinated rat blood or chopped 15 day old rat embryo. They were exposed to saturated p-dichlorobenzene vapors for two to three hours daily for 14 days, and then injected with 0.2 cc of leukemia cells.

Of the 40 animals in the immunized group exposed to p-dichlorobenzene, 67.5% had tumors. An immunized group not exposed to p-DCB recorded 20.5% tumors while the control (no immunization) group exhibited an 84.2% tumor incidence. It would appear that p-dichlorobenzene modified the induced resistance of the rats to the leukemia. However, there is not sufficient evidence to state that there are possible immunosuppressant effects resulting from exposure to p-dichlorobenzene.

In the two studies by Hollingsworth, et al. (1956, 1958) described earlier, the investigators did a cursory survey for tumor occurrence during the subchronic exposure of rats, rabbits and guinea pigs. No tumors were reported in any species after exposure to either substance (o- and p-DCB). Little confidence can be placed in these results, however, as the conditions of study were inadequate for evaluating carcinogenic or mutagenic potential.

The National Toxicology Program (NTP) has completed carcinogenicity bioassays in two species of rodents (rat and mouse) for ortho- and para-dichlorobenzene. The results of the chronic gavage studies on the ortho- isomer were presented to the NTP Board of Scientific Counselors in a draft report (NTP, 1982). The Board has not approved the report as yet. Nevertheless, the results are summarized below. The chronic gavage studies for p-DCB are complete, but the results have not been presented as yet. The results of studies conducted in support of the chronic experiments with both isomers are presented in this section as well.

Ortho-dichlorobenzene

Mice

Two 14-day studies with o-DCB were conducted in B6C3F1 mice as part of the prechronic test phase of the NTP bioassay (Battelle-Columbus, 1978a,b). These studies were designed to determine doses for the three-month subchronic toxicity study. No acute toxicity study was conducted. In the first study, doses of 0, 250, 500, 1000, 2000 or 4000 mg/kg in corn oil were administered daily by gavage to six groups of five mice of each sex for 14 successive days (Battelle-Columbus, 1978a). By the end of the study, 47 of the 60 mice had died, leaving 5 male and 8 female survivors. All mice in the top two dose groups died by day 3. In addition, 1 male mouse in the control group died on day 4.

Gross observation of mice dying early revealed that the animals had pale livers. This effect was seen as early as day 2 in the 4000 mg/kg group and by day 7 in the 250 mg/kg females. Other gross lesions observed in all dose groups included reddened lung areas and yellow-green or red small intestines. Histopathological changes in the six male mice examined were characterized by moderate to severe hepatic centrilobular necrosis at the 500 mg/kg/ level and milder (in one) or none (in two) at the 250 mg/kg level. One mouse given 500 mg/kg exhibited lymphoid necrosis of the spleen, while another showed marked lymphoid depletion of the thymus. Seven females were subjected to histopathological examination. One mouse given 1000 mg/kg survived to the end of the study but showed no pathological changes. Of the three mice examined from the 500 mg/kg group, one of two early fatalities exhibited no abormalities while the other had moderate lymphoid depletion of the spleen. The one survivor had no lesions. At 250 mg/kg, one early fatality exhibited mild centrilobular hepatic necrosis, the other, no histopathological changes. The one survivor examined showed no pathology.

Since a no-effect dose level was not determined from the initial experiment, it was decided that a follow-up study would be conducted, using a range of doses lower than employed in the first experiment. The protocol was identical to that of the first study; however, the oral doses of o-DCB selected were 0, 30, 60, 125, 250 or 500 mg/kg (Battelle Columbus, 1978b). In this study, only two early deaths occurred: one male died in the 500 mg/kg group on day 3 and one female died in the 125 mg/kg group on day 8.

Body weight changes in the treated animals were not markedly different from the controls. During the first three days of the study, all mice in the highest dose group exhibited labored breathing, rough coats and watery eyes. The signs then disappeared. Gross examination was conducted on all animals. The male mouse dying on day 3 had a pale, mottled liver, enlarged stomach and reddened small intestine. At the termination of the experiment, histological examination of livers from four males and four females from the 500 mg/kg dose group revealed no changes in two males, mild focal necrosis in one and mild focal necrosis as well as cytomegaly, karyomegaly and chronic moderate multifocal granulomatous hepatitis in the fourth male. Among the four females, one exhibited moderate focal necrosis, another showed mild focal necrosis and the other two had mild centrilobular degeneration with cyto- and karyomegaly. These changes were judged to be treatment-related. On the basis of the results of this second study, it was recommended that the subchronic study in the mouse be performed at the same dose levels used in this 14-day study.

The subchronic toxicity gavage study with o-DCB in mice was conducted to assist in dosage selection for the 104-week chronic study (Battelle Columbus, 1978c). The doses used were as detailed above (0, 30, 60, 125, 250 or 500 mg/kg/day). Treatment groups consisted of five animals of each sex. Single gavage doses of the compound in corn oil were administered 5 days/week for 13 weeks. Weekly individual body weights and cage food consumption rates were monitored. Animals were sacrificed on day 92 or 93, with full necropsy, recording of organ weights and histological examination of various tissues. Special studies also were performed near or at the end of the exposure period. These included: urinalysis in the highest dose group and controls, hematology and clinical chemistry. Organ/body weight ratios were calculated and urine and liver porphyrin determinations made.

During the study, lethargy and rough coats were observed in both sexes at the four highest doses. However, by the final week on test, only animals of both sexes at the 500 mg/kg dose and males at 250 mg/kg showed these signs. Body weight gain was affected in males at 500 and 250 mg/kg (lower by 49% and 18%, respectively, when compared with controls). Female mice receiving 500 mg/kg/day exhibited a weight gain of 62% of controls. No other groups showed differential weight gains of less than 10% when compared with controls.

Hematological parameters evaluated included hemoglobin, hematocrit, total and differential white counts, red cell and platelet counts, mean corpuscular volume and reticulocyte counts. The investigators did not perform any statistical analyses on these data, but claimed that no clinically-significant treatment-related changes occurred. Apparent increases in white cell counts of treated males when compared with controls were attributed to relatively low counts among the controls. It was suggested that these counts were below those typically observed in that laboratory and others ($3.4 \times 10^3/mm^3$ in controls vs. $5.4-6.6 \times 10^3/mm^3$ in the treated groups). Since the individual data were not made available, the statistical tests cannot be done to determine whether there were significant differences between the controls and the treated groups. In addition, information on "normal" counts is not available for consideration. But, since there is at least anectodal evidence of a possible relationship between exposure to ortho-dichlorobenzene and leukemias in humans, it would be prudent to evaluate these results in greater depth.

Blood samples were analyzed for alkaline phosphatase, SGPT and gamma-glutamyl-transpeptidase (GGTP). No GGTP was detected in any sample. Statistically significant dose-dependent changes in alkaline phosphatase did not occur, although increased levels were noted in males receiving 125 and 250 mg/kg/day. SGPT levels in the two surviving males receiving 500 mg/kg/day were increased significantly over control, due to a high value in the animal exhibiting the hepatocellular necrosis. The other animal showed no hepatic histopathological changes.

The following parameters were monitored in mice in the highest dose group and controls during urinalyses: pH, glucose, protein, bilirubin, ketones, occult blood, specific gravity and creatinine. The report stated that the volume of urine collected from treated animals, especially the males, was generally greater than that collected from controls. The individual data were not available to corroborate this conclusion. No record of fluid intake was kept during the study. Decreases in specific gravity and creatinine were noted, reflecting dilution due to increased urine output. No other compound-related effects were observed.

Uroporphyrin levels in treated males were generally higher than in the controls. In females, coproporphyrin levels were higher in 7 treated animals than in controls (1876 ± 1674 S.D. vs.

332 ± 210 S.D.). Coproporphyrin levels in treated males and uroporphyrin levels in females were not different from controls. Sex differences were seen in liver protoporphyrin levels, as a dose-dependent increase was observed in the females but not in the males.

Organs weighed were: heart, lung, kidney, testis, spleen, thymus, brain, ovary and uterus. While no statistics were done, it appeared that relative liver weights were increased in the highest dose males and females. An increase of lesser magnitude was seen in females receiving 250 mg/kg/day. No other differences were noted.

All of the above-mentioned tissues and skeletal muscle from control and highest dose (500 mg/kg) animals were examined microscopically. The liver, thymus, heart, spleen and thigh muscle were examined from animals in the 250 mg/kg group while only livers from the 125 mg/kg group were examined. Lesions were observed in all tissues from the highest dose (500 mg/kg) animals. These were considered to be treatment-related, since they were absent or occurred less frequently in the controls.

Livers of animals in the 500 mg/kg group exhibited significant centrilobular necrosis, hepatocellular necrosis and degeneration, and deposition of yellow-green to golden pigment which was considered to be hemosiderin. The heart showed multiple foci of mineralization in the myocardial fibers. Skeletal muscle also exhibited mineralization as well as necrosis and myositis. Some animals had lymphoid depletion of the spleen and thymus. One female exhibited lymphocyte necrosis in the spleen.

Among the animals receiving 250 mg/kg, only hepatocellular necrosis was noted in two males, with pigment deposition in one male and hepatocellular degeneration in one male. No lesions were noted in the group treated with 125 mg/kg.

The results of this study suggest that an oral no-effect level of 125 mg/kg/day can be identified in mice over 13-week exposure period. Doses of 60 and 120 mg/kg/day were selected for use in the 104-week chronic study.

A draft NTP Technical Report on the bioassay of ortho-DCB in mice and rats was presented to the NTP Board of Scientific Counselors on September 22, 1982. This document has not become a final report. Therefore, the text that follows represents only a preliminary assessment of the data from the bioassay.

One phase of the bioassay was conducted in mice by administering 60 or 120 mg/kg doses of o-DCB in corn oil, five days/week for 104 weeks. Groups of 50 males and 50 females comprised each dosage group and a vehicle control group, as well. The control group received equivalent volumes of corn oil on the same schedule as the treated animals.

No difference in survival rates were observed between the treated and control groups of either sex. There was no evidence of compound-related nonneoplastic liver lesions.

Statistically significant positive trends in the incidence of malignant histiocytic lymphomas occurred in mice of both sexes ($P<0.05$). However, the incidence of total lymphomas of all cell types was not significantly increased above control. The draft reports states that "since the histiocytic lymphoma is a controversial diagnosis among different pathologists and since all types of lymphomas have the same histiogenesis, an increase in this specific type of lymphoma in the absence of an increase in the total incidence of all types of lymphoma is not considered to be biologically significant."

An increase in alveolar/bronchiolar carcinomas was observed in male mice (control=4/50 (8%);low dose=2/50 (4%);high dose=10/50 (20%)). This increase was shown to be significant when analyzed by the Cochran-Armitage test, but not by the life-table or incidental tumor test. Thus, this increase was discounted because the combined incidence of males with alveolar/bronchiolar adenomas or carcinomas was not significantly greater than controls by any of the three tests (control=8/50 (16%);low dose=8/50 (16%);high dose=13/50 (26%)).

Male mice also exhibited a significant decrease in hepatocellular adenomas at the high dose (control=8/50 (16%);low dose=5/49 (10%);high dose=2/46 (4%)). This decrease was accompanied by a negative dose-response trend using the Cochran-Armitage test. When total incidence of adenoma or carcinoma in the high dose males was evaluated, this decreased incidence was statistically significant only by the life-table test (control=19/50 (38%);low dose=14/49 (29%);high dose=11/46 (24%)).

The preliminary assessment suggests that, under the conditions of this bioassay, ortho-dichlorobenzene was not carcinogenic in the B6C3F1 mouse of either sex. However, the maximum tolerated dose was probably not achieved in the study.

Rats

A 14-day study with o-DCB also was conducted in Fischer 344 rats as part of the prechronic test phase of the NTP bioassay (Battelle Columbus, 1978d). Oral gavage doses of o-DCB in corn oil of 60, 125, 250, 500 and 1000 mg/kg were selected. Five animals of each sex were placed in one of five treatment groups or one control group. All of the 10 rats in the 1000 mg/kg group died early, the males by day 4 and the females by day 5. No other early deaths occurred. The percentage body weight gain decreased with increasing dosage in both sexes. Males at 250 mg/kg showed an 11% deficit, those in the 500 mg/kg group, a 16.6% differential. Only females at 500 mg/kg showed a statistically significant lack of body weight gain exceeding 10% (11.8%).

No tissues from animals dying early were examined histologically. Rats in the highest dose group (1000 mg/kg) exhibited, upon gross examination, pale and yellow livers, yellow and/or green or red colored contents in the small intestine, similarly-colored fluids in the urinary bladder, red fluid in the cecum and congestion of the vasculature of the brain. The liver lesion was interpreted to reflect hepatotoxicity. No toxic lesions were observed in other dosage groups during gross examination. Tissues from two males and two females receiving 500 mg/kg/day were examined microscopically. No lesions indicative of toxicity were observed.

Based upon the results of the 14-day study, doses of 0, 30, 60, 125, 250 and 500 mg/kg/day in corn oil were administered to Fischer 344 rats in the subchronic study (Battelle Columbus, 1978i). Controls received corn oil. Groups consisted of five animals of each sex. Dosing was done 5 days/week for 13 weeks. Special studies, as described for o-DCB in mice and p-DCB in both species, also were conducted in this study. No statistical analyses were performed on the data from these special studies, except for determination of group means and standard deviations.

Of the animals used in this study, only four died early: one male each in the 30 mg/kg (week 11) and control (week 10) groups and two females in the highest dose group (weeks 6 and 9). Nine males in the 250 mg/kg group completed the study because the tenth was found to be a female during week 10 and was removed from the group.

Food consumption did not vary more than 10% in treated groups when compared with controls (down 1.5 mg/day in the highest dose males). There was a dose-dependent decrease in body weight gain in both sexes. At 250 mg/kg and 500 mg/kg, this differential exceeded 10%. No striking differences in hematological parameters were noted, but without statistical analysis, it is difficult to determine if the changes are truly statistically non-significant. Those parameters which may have been altered significantly in the highest dose males were hematocrit (down 5%) and red cell count (down to $8.57 \pm 0.25 \times 10^6$ cells/mm^3 from an average of $9.42 \pm 0.53 \times 10^6$ cells/mm^3 in the control group). There appeared to be a trend towards an increase in platelet count. This increase was directly proportional to increasing dose in the females (with levels of 300,000 in the controls to 365,000-600,000 as the dose increased). Values for the 250 mg/kg group did not fall into this sequence. This apparent discrepancy may have been due to what appeared to be a lower-than-normal count in the controls.

Of parameters measured in the clinical chemistry analyses, cholesterol levels were increased in males in the 250 and 500 mg/kg groups and in females in the 125, 250 and 500 mg/kg groups. Triglyceride levels dropped in the highest dose males. The combined alpha-globulin fraction appeared to be increased in males receiving 250 and 500 mg/kg and in females treated with 500 mg/kg. Of the parameters evaluated in the urinalyses, urine volume was altered significantly. Output increased an average of 157% in treated males and 187% in treated females, when compared with their respective controls. Decrease in urine creatinine accompanied the dilution occurring with the increased volume output.

Uroporphyrin and coproporphyrin levels in the urine showed an increase in the highest dose animals (3-6 fold). Liver protoporphyrin levels were not altered significantly at any dose level. Thus, there appeared to be abnormal excretion but not retention of the porphyrin.

Organ weights and organ/body weight ratios were determined. In rats of both sexes receiving 250 or 500 mg/kg, relative liver weights were increased. The liver/body weight ratios in the other treatment groups did not appear to differ from the controls. Organ: body weight ratios for spleen, kidney, testis, ovary, uterus, thymus, brain and heart did not appear to have been altered.

No consistent lesions were noted upon gross examination at necropsy. Microscopic examinations were performed on tissues of all animals in the control and 500 mg/kg groups and on the thymus, liver and kidney of the animals from the 125 and 250 mg/kg groups. A moderate degree of centrilobular hepatic necrosis was seen in the livers of the highest dose rats which died early. Of the survivors in that group, most showed liver lesions, either centrilobular degeneration or necrosis of individual hepatocytes. This necrosis was characterized by randomly scattered cells that were pyknotic or karyolytic, with shrunken, dark red cytoplasm. Some of the 500 mg/kg group males also exhibited renal tubular degeneration and lymphoid depletion of the thymus. These latter lesions were not seen in other treated groups or controls. However, hepatocellular necrosis was observed in the 250 mg/kg group (4/10 males and 5/10 females). In one female treated with 125 mg/kg, the liver was shown to contain granules which were considered to be hemosiderin since they stained PAS- and Perls positive.

The liver lesions observed in the 250 and 500 mg/kg groups were considered by the investigators to be dose- and treatment-related, and probably life-threatening, as were the renal and thymic lesions in the high dose group. The reviewing pathologist recommended that a Maximum Tolerated Dose (MTD) of 125 mg/kg (the apparent no-effect level) be set for both sexes of rats in the 104-week chronic study. Ultimately, doses of 60 and 120 mg/kg were chosen for this study.

As mentioned above, this assessment of the effects of o-DCB in the bioassay is preliminary, pending acceptance by the NTP Board of Scientific Counselors.

As with the previously-described chronic study in mice, groups of 50 male and 50 female rats received 60 or 120 mg/kg doses of o-DCB in corn oil, five days/ week for 104 weeks. Controls, (also 50 of each sex) received an equal volume of corn oil on the same schedule.

There was no significant difference in the survival rates of female rats of either treatment group or low dose males when compared with the controls. There was a significant decrease in the survival of the males at the high dose ($P<0.001$). However, several of the males dying before the end of the study were found to have corn oil or o-DCB in corn oil in their lungs (3 in the control group, 8 at the low dose and 12 at the high dose). Therefore, gavage trauma may have contributed to their deaths. The report suggests that the lower survival rate among the high dose males may not reflect that the maximum tolerated dose was exceeded. In fact, as was seen in the mice, there was no increase in the incidence of nonneoplastic lesions of the liver in rats receiving either dose of o-DCB, again suggesting that a higher dose may have been tolerated.

The low dose males showed a significant increase in the incidence of adrenal pheochromocytomas when compared with the control group by life-table analysis (control=9/50 (18%);low dose=16/50, (32%);high dose=6/49 (12%)). The report concludes that since this incidence was shown to be significant only by one of the three statistical tests, and the tumor incidence was not dose-dependent and there were no malignant pheochromocytomas, the increase seen in the low dose group was not related to treatment with o-DCB.

Interstitial cell tumors of the testis in the males also occurred with a significant positive trend when analyzed by the life table test (control=47/50 (94%);low dose=49/50 (98%);high dose= 41/50 (82%)), but with a significant negative trend when analyzed by the Cochran-Armitage test. The reports states that "since this tumor is not considered to be life-threatening, this increase detected by the life table test was discounted."

Preliminary assessment of these data suggests that, under the conditions of this bioassay, ortho-dichlorobenzene was not carcinogenic in the Fischer 344 rat of either sex, but that the maximum tolerated dose may not have been achieved.

Para-dichlorobenzene

Mice

A 14-day dosing study was conducted in B6C3F1 mice to determine doses for the 13-week subchronic toxicity study (Battelle-Columbus, 1978e). Doses of 250, 500, 1000, 2000 or 4000 mg p-DCB/kg in corn oil were administered to groups of five males and five females for 14 consecutive days. Controls received corn oil only on day 1 and were untreated the remaining 13 days. All mice receiving 4000 mg/kg died by day 3. At 2000 mg/kg, four males died by day 7 and two females by day 8. At 1000 mg/kg, four males and two females died by day 8. At 500 mg/kg, four males died by day 8, but all females were dead by day 5. At 250 mg/kg, three males died by day 7, three females by day 6. Among the controls, two males and one female died on day 3, presumably as a result of gavage trauma.

A number of lesions were noted upon gross necropsy in both sexes at all dose levels. These changes included: livers of abnormal color (either yellow or tan or reddish-chocolate), soft or mushy small intestines (yellow-green through pink or red to black in color) and pink to bright red lungs. Occasionally, the kidneys were pale and the mandibular salivary glands were enlarged and chalk-white in appearance. According to the investigators, no lesions were seen microsopically in either sex or at any dose level which indicated significant toxicity.

Since the study did not establish a no-effect level, a second 14-day oral dosing study was performed (Battelle Columbus, 1978g). Male and female mice were dosed by gavage with 60, 125, 250, 500 or 1000 mg p-DCB/kg in corn oil for 14 consecutive days. The controls in this study were untreated. Only one early death occurred (a male in the 125 mg/kg group), purportedly due to gavage trauma. Only the 500 mg/kg females showed a decreased body weight gain (10.9% lower than control). No gross pathology was noted at necropsy. No microscopic examination was performed.

On the basis of these two 14-day studies, doses employed in the first subchronic study were 600, 900, 1000, 1500 and 1800 mg p-DCB/kg/day, 5 days/week for 13 weeks (Battelle Columbus, 1979a). Ten males and ten females were assigned to each group. Special studies included: urine and liver porphyrin determination, calculation of organ/body weight ratios, urinalyses, clinical chemistry and hematology.

Twenty-six mice (12 males and 14 females) died before the end of the study. Seven males and females in the 1800 mg/kg group died early, most within the first week. At 1500 mg/kg, three males and five females died early. One male in the 1000 mg/kg group and one control male died during the last week.

No effects on food consumption were noted in any group. Differential body weight gains in treated males in all groups were significantly different from controls. These differences ranged from 50% at 1500 mg/kg to 22.9% at 1000 mg/kg. The differences did not show a dose-related trend. Females showed a differential weight gain of 39% at 600 mg/kg, with lesser changes as the dose increased. Since food consumption was not altered, the weight gain reductions were apparently unrelated to dietary intake.

The hematological parameters which were evaluated included: hemoglobin, hematocrit, total and differential white cell counts, red cell counts, mean corpuscular volume, platelet and reticulocyte counts. No statistical analyses were performed on these data. However, the investigators stated that there appeared to be no significant differences between treated groups and controls. However, a cursory review of the group data suggest that there may have been a significant decrease in platelet counts in the high dose males (445,000 ± 8,700 vs 691,900 ± 209,200 for controls), and a significant increase in platelet count in surviving females at the two highest doses (707,400 ± 41,000 at 1500 mg/kg, 707,500 at 1800 mg/kg vs 492,000 ± 86,631 for controls). This latter observation may be the result of a lower than normal platelet count among the control animals.

The following analyses were performed on blood samples drawn at the time of sacrifice: serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, gamma-glutamyltranspeptidase (GGTP), bilirubin, cholesterol, triglycerides, blood urea nitrogen (BUN), glucose and total protein. While no statistical analyses were performed on the data, it appeared that triglycerides were increased in males at the two highest dose levels (305 ± 40 S.D. mg/dl in the 1800 mg/kg group, 262 ± 24 mg/dl in the 1500 mg/kg group vs. 174 ± 33 mg/dl in the controls). The cholesterol levels increased in all males except those in the 600 mg/kg group (244 ± 16 mg/dl at 1800 mg/kg, 179 mg/dl at 1500 mg/kg, 151 ± 21 mg/dl at 1000 mg/kg and 160 ± 21 at 900 mg/kg vs. 95 ± 8 mg/dl at 600 and 91 ± 11 mg/dl in the controls). Total protein appeared to be increased in males in the 1800 mg/kg group (7.1 ± 0.2 g/dl compared with 5.7 ± 0.2 g/dl in the controls). Since there were no increases in hemoglobin or hematocrit, this increase likely was due to an actual, rather than a relative, increase in blood proteins.

Both SGPT and bilirubin decreased in females in the two highest dose groups. No control values in females were recorded for cholesterol, triglycerides, BUN, glucose or total proteins. Thus, it is difficult to determine whether or not the increase in cholesterol values in these groups was significantly greater than control, as would be suggested by the dose-related upward trend observed in the males.

Urinalysis parameters included: pH, protein, glucose, ketones, bilirubin, occult blood, specific gravity and creatinine, as well as uroporphyrins and coproporhyrins in the controls and two highest dose groups.

Ketonuria was noted in one of two pooled groups of males at the 1,500 mg/kg dose level and in both pooled groups of females at that dose. None was observed in urine of mice at other doses or in the controls. According to the investigators, this occurrence may have been due to severe depression in body weight gain differential in males. However, this postulate does not hold for the females, since the weight gain differential was not observed. The investigators suggested that this observation was due to contamination of the urine samples. As was seen in the o-DCB studies, there was increased urinary output. This finding was especially notable in the males.

Urinary coproporphyrin levels in both sexes appeared to increase significantly at the 1500 mg/kg/dose level. This increase was seen only in females at the highest dose. Dose-dependent increases in liver protoporphyrin occurred in both males and females. However, since no statistics were applied to the data, one cannot identify the lowest dose level at which significant increases occurred.

Of the organ/body weight ratios calculated, those for the liver were increased at all dose levels for both males and females. In addition, relative uterine weights of females decreased in a dose-related manner, with the greatest decrease in the ratio observed in mice receiving 1,800 mg/kg. No differences were apparent for lung, heart, kidney, spleen, thymus, brain, testis or ovary.

The only anomalies noted during necropsy were some pale and/or yellow livers and pale kidneys in the highest dose group. No hemosiderin was noted in any liver or kidney of mice from any dosage group.

A number of lesions were noted upon histological examination. These included lesions of the spleen, thymus, bone marrow and lymph nodes among animals from the two highest dose groups. These changes were considered to be treatment-related. Hepatocellular changes, which were seen in treated animals of all groups, were not seen in the controls. Lymphoid depletion of the spleen, lymphoid necrosis in the thymus and hematopoietic hypoplasia in the spleen and bone marrow were observed in mice from the 1500 mg/kg group which died before the end of the study, but not in those who survived to the end. The hepatocellular changes observed in the various groups included karyomegaly, cytomegaly, and occasional nuclear pleomorphism of centrilobular hepatocytes. The cytoplasm of these enlarged cells was grainy, sometimes hazy and amphophilic. These changes were dose-related.

Since the hepatocellular changes were seen in animals from all treated dose groups, a no-effect level could not be established from the study results. The changes observed at 600 mg/kg could be characterized as minimal. Subsequently, a second subchronic study was performed in the attempt to establish the maximum tolerated oral dose for the chronic study and a no-effect dosage level for subchronic exposure (Battelle-Columbus, 1980a). In this second study, target doses of 75, 150, 300, 600 and 900 mg p-DCB/kg/day were chosen. As shown later, because of an error in the preparation of the doses, those actually administered were 84.4, 168.8, 337.5, 675 or 900 mg/kg/day. These doses were given to groups of 10 male mice and 10 female mice by gavage five days/week for 13 weeks. The protocol was otherwise identical to the first subchronic study, except that no special studies were performed. During the study, three males and seven females died early. These deaths were not dose-related, but rather attributable to gavage trauma. No significant differences were apparent in dietary consumption or relative weight gains between treated and control animals of either sex.

During gross necropsy, no consistent observations were made which were considered significant. Microscopically, there was a significant number of mice in the two highest dose groups exhibiting centrilobular to midzonal hepatocytomegaly. Few changes of this type were seen in animals treated at 337.5 mg/kg/day. The investigators determined that 337.5 mg/kg/day was the maximum tolerated dose for this duration of exposure.

(104-week results to be added later)

Rats

Fourteen-day and 13-week subchronic gavage studies also were conducted in Fischer 344 rats as dose-range finding efforts preliminary to the 104-week chronic bioassay for p-DCB in this species. The first 14-day study employed doses of 60, 125, 250, 500, or 1000 mg/kg/day p-DCB in corn oil (Battelle Columbus, 1978f). Doses were administered to five males and five females per group. Controls were untreated.

Only one early death was recorded, a male at the 125 mg/kg dose level on day 8. Body weight gains were slightly suppressed in the males, with the greatest difference being seen at the highest dose (9.2%). No gross or histopathological changes were observed. Because of the absence of significant signs of toxicity, it was decided that the study be repeated at higher dose levels.

A re-run of the 14-day study was conducted according to the protocol of the first study. In this study, however, doses of 500, 1000, 2000, 4000 or 8000 mg/kg/day were administered in corn oil to the rats (Battelle Columbus, 1978g). Again, controls were untreated. No clinical pathology studies or histological examinations were done.

By day 3, all animals in the groups receiving 2000, 4000 or 8000 mg/kg had died. Gross pathology included pale livers, discolored lungs and intestines, enlarged mandibular lymph nodes and fluid discharge from the eyes and nose. One male rat receiving 1000 mg/kg died on the first day, apparently due to gavage trauma. Four females in that dose group died on day 1 (1), day 4 (1) and day 5 (2). Those dying on day 5 showing slightly congested livers. One female receiving 500 mg/kg died on day 13 of gavage trauma.

At these higher doses, evidence of depressed body weight gain appeared. Among males, those receiving 1000 mg/kg/day showed a 22% reduction from controls; those receiving 500 mg/kg/day showed a 24% reduction. No significant differences were seen in the females at any dose.

From the results of the two 14-day studies, it was determined that the first subchronic gavage study in the Fischer 344 rat employ dose levels of 300, 600, 900, 1200 or 1500 mg p-DCB/kg/day (Battelle Columbus, 1979b). The protocol included use of ten animals/sex/dose or control group. Animals received doses of p-DCB in corn oil, five days/week for 13 weeks. Vehicle controls received corn oil alone. In addition to gross necropsy and histological examination, additional special studies were performed as described for the subchronic studies on o-DCB. These included: clinical chemistry and hematology on blood samples drawn the day of sacrifice, urinalyses on animals in the 1500 and 1200 mg/kg groups and controls, porphyrin analysis in urine and liver of the same groups and calculations of organ/body weight ratios. Again, no statistical analysis of these data was performed.

Eight early deaths occurred in the 1500 mg/kg males, and five early deaths occurred in males at 1200 mg/kg. One male receiving 900 mg/kg died during week 1, and one control male during week 10. Among females, 9 of 10 receiving 1500 mg/kg died early while one of 10 at 1200 mg/kg and two of 10 at 900 mg/kg died before the end of the study. The males tended to die earlier than the females.

Food consumption in the treated groups varied little from control. The one remaining male at 1,500 mg/kg showed a slight increase compared with controls. Male rats dosed with 300 mg/kg averaged slightly less food consumption than controls. A dose-dependent depression of body weight gain was seen in both sexes. In females, a greater than 10% difference was seen at the 900 mg/kg level and above. All treated males exceeded the 10% differential when compared with controls.

Of the hematological parameters analysed (hemoglobin, hematocrit, mean corpuscular volume, red cell, reticulocyte and platelet counts, total and differential white cell counts), the hemoglobin and hematocrit levels appeared to be lower in the two surviving males at 1,500 mg/kg. Hemoglobin levels were 17.6 ± 0.6 G/dl in controls vs. 15.3 ± 0.2 G/dl in the treated rats. These two males also exhibited mild anemia with average red cell counts of $8.85 \pm 0.22 \times 10^6$ cells/mm^3 compared with control levels of $10.03 \pm 0.36 \times 10^6$ cells/mm^3. Mean corpuscular volume also was decreased slightly (51 ± 2 in controls vs. 48 ± 0 in the treated rats). The investigators reported that no histological evidence of bone marrow changes were apparent in these two rats, although one other male and five females treated with this dose did exhibit bone marrow hypoplasia. The single surviving high dose female showed an increase in hemoglobin, lowered white cell count, increased red cell count and decreased mean corpuscular volume when compared with controls. However, one cannot establish the toxicological significance of these observations, since they occurred in a single animal.

Clinical chemistry analyses on blood drawn at sacrifice included: GGPT, alkaline phosphatase, bilirubin, cholesterol, triglycerides, total protein, blood urea nitrogen, glucose and globulin fractions. Of these parameters, there was evidence of a possible dose-related increase in alkaline phosphatase in females, with a substantial increase in the lone survivor at the highest dose. Trends also appeared in both sexes as an increase in cholesterol levels with increasing dose and an increase in albumin levels at the highest doses. The total protein increases seen at the higher doses likely are due to the increase in the albumin levels.

The investigators reported no significant changes in the parameters measured in the urinalyses (pH, protein, glucose, creatinine, ketones, bilirubin, occult blood and specific gravity). As previously described for o-DCB, urine volume of rats treated at the higher dose levels was significantly increased: 246% in males and 142% in females receiving 1200 mg/kg when compared with controls. The few survivors from the 1500 mg/kg group had urine volumes 6-10 times greater than control levels. Since drinking water intake was not monitored, one cannot speculate on the consequences of this alteration in output.

Porphyrin levels in urine and liver also were monitored in the two higher dose group and control males. Significant increases in both coproporphyrin and uroporphyrin levels in the urine were observed. A similar increase was seen in the urine of the surviving highest dose female. No data for survivors at 1,200 mg/kg were presented. Liver protoporphyrin levels apparently were not increased in either sex. On the contrary, the levels in the survivors at the highest dose were depressed somewhat. Therefore, there was no retention of porphyrins in treated animals, just as was reported for o-DCB.

Several organs were weighed at the time of sacrifice. These included: heart, liver, kidney, uterus, ovary, testis, brain, thymus and spleen. The relative liver weights were increased clearly in animals of both sexes at all doses except at the lowest dose (300 mg/kg). An increase at this dose level was seen in females, but it may not have been statistically significant. Dose-related increases in brain/body weight ratios were observed. Absolute uterine weights decreased with treatment, but the organ/body weight ratios were not altered significantly.

Histological examination was performed on tissues from animals in the control and three highest dose groups. The kidneys and lungs from males treated at 300 and 600 mg/kg also were examined.

Retinal atrophy was seen in almost all of the animals including controls, the degree of severity of which was inversely related to the time of death. Thus, those dying early showed few signs; those dying later had severe bilateral atrophy.

Pulmonary lesions related to the gavage procedure were seen in animals of all tested groups. Presumably, aspiration of the test material occurred.

Histological changes of significance included degeneration and necrosis of the hepatocytes, hypoplasia of bone marrow, lymphoid depletion of the spleen and thymus, epithelial necrosis and villar bridging of the mucosa of the small intestine and epithelial necrosis of the nasal turbinates. These changes occurred only in animals in the two highest dose groups. None of the lesions were observed in animals receiving doses of 900 mg/kg or less. Thus, these changes were considered to be treatment- and dose-related.

Most of the males surviving beyond the halfway point of the study exhibited renal lesions characterized by multifocal degeneration or necrosis of the cortical tubular epithelium. An amorphous eosinophilic material was often present in the lumen of these tubules. Some thickening of the basement membrane of these cells was visible. Occasionally, dilatation of some tubules could be observed at the corticomedullary junction. These tubules also had degenerated epithelia and were filled with material similar to that described above.

The investigator describing the pathology stated that the renal lesions are not unusual for male rats of this strain. However, he would have expected to have seen lesions of some magnitude in the control animals, and to a lesser degree in treated ones. Thus, he concluded that while the lesion was not clearly dose-related, it might be at least partially treatment-related and for this reason, a no-effect dose level could not be established from the results of this study. Thus, it was decided that the subchronic gavage study in rats would be repeated, employing lower dose levels of 37.5, 75, 150, 300 or 600 mg/kg/day (Battelle Columbus, 1980b). The protocol employed was identical to that described above for the first subchronic study, except that no special studies were done. A few early deaths occurred during the second study. All were attributed to gavage trauma.

No significant differences in food consumption were observed. No dose group had a greater than a 10% differential body weight gain when compared with controls. Histological examination was performed on all control and 600 mg/kg dose animals, as well as all early death animals, and kidneys from males at the two lower doses.

Microscopically, some pulmonary changes were noted which were attributed to aspiration of the gavaged material. There also was an increased incidence and severity of renal cortical degeneration in males receiving the two higher doses, as had been observed in the earlier study. Females showed no significant changes at any dose level. Some rats also exhibited myocardial degeneration and lymphoid hypoplasia in lung tissue. These changes were characterized as being normal for this strain and age of rat. The reviewing pathologist suggested that the MTD for males be set at 150 mg/kg and for females at 600 mg/kg for the chronic study. Therefore, Battelle Columbus proposed doses for the chronic study of 75 and 150 mg/kg in males, 300 and 600 mg/kg in females. Tracor Jitco suggested 150 and 300 mg/kg in males, 300 and 600 mg/kg in females.

(104-week study to be added later.)

Other Carcinogenicity Studies

Long term inhalation studies with p-DCB have been conducted in mice and rats (Riley, 1980a,b). Groups of Swiss strain mice (75 males and 75 females per group) were exposed to airborne concentrations of 0, 75 or 500 ppm p-DCB five hours/day, five days/week for 57 weeks for all female groups and the 500 ppm males and for 61 weeks for the 0 and 75 ppm males (Riley, et al., 1980b). Males were sacrificed at these times. Females were held unexposed until sacrifice after 75 or 76 weeks.

The original objective of this study was to assess the chronic toxicity and carcinogenic potential of p-DCB in the mouse. However, because of fighting among the males during the early stages of the study and a high background incidence of respiratory disease in both sexes resulting in high mortality rates, the investigators felt that this objective was not attained satisfactorily.

A similar long term inhalation study was conducted in Alderly Park Wistar-derived albino rats (Riley, et al., 1980a). Groups of animals (76-79 animals/sex/group) were exposed to airborne concentrations of 0, 75 or 500 ppm p-DCB five hours/day, five days/week for 76 weeks. Survivors at this time were left unexposed for 36 additional weeks. Interim sacrifices (5 animals/sex/group) were conducted at 26, 52 and 76 weeks. Body weights were determined weekly until week 13 and monthly thereafter. Food and water consumption was monitored biweekly until week 15, then every six weeks thereafter. Urinalyses, clinical chemistry and histopathological parameters were analyzed in the rat study. Hepatic aminopyrine demethylase activity also was measured at the 52 week sacrifice. Organ weights were measured at each sacrifice date.

No consistently significant effects on mortality rate, food and water consumption, body weight gain, clinical chemistry, hematology or histopathology were observed in either sex, at either exposure dose or at any of the sacrifice times when compared with controls at the same times. Group mean body weights in the high dose females were significantly depressed from Weeks 4-38 except at Week 10, but were not significantly different at Week 50 or later. Increases in liver and kidney weights (both sexes at Weeks 76 and 112), heart and lung weights (both sexes at Week 112) and urinary protein and coproporphyrin output (in males) were noted in animals exposed at 500 ppm. No increases in tumor incidence or types were produced at either exposure level in either sex. The investigators concluded that, under the conditions of this study, p-DCB was not carcinogenic to rats at exposure levels up to 500 ppm. An increase in hepatic hyperplasia was seen in females treated at both doses but not in controls or treated males. Hemosiderosis was increased in treated males at both doses, but not in females. Focal chronic hepatitis with infiltration was increased in all treated animals, but significantly so only in the animals exposed at 500 ppm. A slight increase in myocardial calcification was seen in high dose males. Adrenal hyperplasia was increased only in the low dose males. The investigators concluded that non-neoplastic changes of a minor nature occurred in the high dose animals, but were absent or insignificant in the low dose animals.

VI. HEALTH EFFECTS IN HUMANS

Most of the poisoning incidents reported in the literature resulted from inhalation. Accidental inhalation can occur either at home or at work. There are several cases, however, when a chlorinated benzene was accidentally or deliberately ingested. Table VI-1 summarizes the available literature for ortho- and para-dichlorobenzene. Again, no reports appear in the literature concerning m-DCB.

o-Dichlorobenzene

One case of sensitization to o-dichlorobenzene was reported for a man who regularly handled window sashes dipped into the compound (Downing, 1939). When o-DCB came in contact with the skin, there was a burning sensation after 15 minutes and for the duration of exposure. The site of exposure showed a reddish hue which increased up to 24 hours later, when blisters formed. A brown pigmentation formed later at the site and persisted for three months. The man was forced to seek alternative employment.

Dupont (1938) described the reactions of a group of sewage workers performing cleaning operations in a sewer at a point directly below a pipe discharging sewage from a dry-cleaning establishment. Inhalation of the fumes caused irritation of the eyes and upper respiratory tract and vomiting. Apparently, no deaths occurred, but no clinical follow-up was described.

An 18-year old female employee of a dry-cleaning shop was admitted to the hospital with severe acute anemia (Gadrat, *et al*., 1962). She had been employed as an ironer in that shop for about 6 months prior to admission. During her employment, she was

Table VI-1

Report of Human Exposure to o- and p-Dichlorobenzene
(Modified from Ware, West, 1977)

Compound	Subject	Exposure	Symptoms	Clinical Report	Follow-up Studies	Reference
o-DCB	Sewage workers	o-DCB effluents from dry-cleaning establishment above sewer	Eye and upper respiratory tract irritation, vomiting	o-DCB intoxication	Not indicated	Dupont, 1938
	47 year old male	o-DCB in dipping solution for window sashes, occupational	Water blisters on face, hands, arms	Eczematoid dermatitis due to o-DCB	Not indicated	Downing, 1939
	18 year old female	Dry-cleaning and dyeing shop	Pallor, tiredness, headaches, vomiting, violent gastric pains	Severe hemolytic anemia: 1.5×10^6 erythocytes/mm^3	10 months later erthyrocytes "excellent" but leucocyte equilibrium showed tendency to neutropenia	Gadrat _et_ _al_., 1962
	40 year old male	1940-1950 occupational exposure to solvent: 80% o-DCB, 15% p-DCB	Weakness, fatigue	Chronic lymphoid leukemia	Treatment ongoing	Girard, _et_ _al_., 1969
	53 year old male	1932-1961, glue containing 2% o-DCB, methyl ethyl ketone & cyclohexane, (no benzene or homologues)	Weakness, fatigue	Chronic lymphoid leukemia, peripheral and abdominal adenopathy, splenomegaly	Died 1968	Girard _et_ _al_., 1969

Table VI-1 (Continued)

Compound	Subject	Exposure	Symptoms	Clinical Report	Follow-up Studies	Reference
o-DCB	15 year old female	Cleaned clothes with products containing 37% o-DCB, (no benzene or toluene)	Initially hospitalized with retroclavicular adenopathy	Acute myeloblastic leukemia	Died 10 months later of 100% peripheral leukoblastosis	Girard *et al.*, 1969
	60 year old male	1930 to 1960 shipping mono, *o*-di- and trichlorobenzene	Weakness, tiredness	Anemia: 3×10^6 erythrocytes/mm^3	Not indicated	Girard *et al.*, 1969
p-DCB	62 year old male	p-DCB in bathroom	Asthenia, dizziness	Light hyperchromic anemia, after 1 month, increase in anemia, hypogranulocytosis	General hematological improvement but increase in hypogranulocytosis at 6 months	Perrin, 1941
	19 year old female	Preparation of p-DCB for 18 months	Asthenia, dizziness, weight loss	Slight anemia, reactional hyperleucocytosis	Not indicated	Petit and Champeix, 1948
	60 year old male	Heavy p-DCB moth ball vapor in house for 3 to 4 months	Weight loss, loose bowels, tarry stools, numbness, clumsiness	Acute yellow atrophy of the liver (confirmed by autopsy)	Developed ascites and died	Cotter, 1953
	Wife of male above	As above	Weight and strength loss, abdominal swelling, jaundice	Acute yellow atrophy of the liver (confirmed by autopsy), splenomegaly	Died 1 year after initial exposure	Cotter, 1953
	36 year old female	p-DCB moth killer in house	Periorbital swelling, intense headaches, profuse rhinitis	Exposure to p-DCB	Symptoms subsided within 24 hours	Cotter, 1953

Table VI-1 (Continued)

Compound	Subject	Exposure	Symptoms	Clinical Report	Follow-up Studies	Reference
p-DCB	34 year old female	Demonstrating p-DCB containing products	Tiredness, nausea headache, vomiting	Subacute yellow atrophy and cirrhosis of the liver	Not indicated	Cotter, 1953
	52 year old male	p-DCB exposure in fur storage plant	Weakness, nausea, blood, vomiting, jaundice.	Subacute yellow atrophy of the liver	4 years later reported "in good health"	Cotter, 1953
	20 year old male (+ 26 workmates)	p-DCB manufacture 1 to 7 months exposure	Loss of weight exhaustion, decrease of appetite	Methemoglobinemia and other blood pathologies	All workers transferred to other working environment	Wallgren, 1953
	53 year old female	12 to 15 year exposure to p-DCB moth balls in house	Cough, progressive dyspnea, fatigue, mucoid sputum	Pulmonary granulomatosis? focal necrosis of liver	Not indicated	Weller and Crellin, 1953
	3 year old male	Played with p-DCB crystals	Cough, listlessness, black urine	Acute hemolytic anemia	Complete recovery	Hallowell, 1959
	19 year old female	4-5 p-DCB pellets ingested daily for 2-1/2 years	Increased patchy pigmentation of skin	Due to p-DCB ingestion. Unsteadiness and tremors on ceasing consumption thought to be psychological, not physiological	Pigment returned to normal	Frank and Cohen, 1961
	21 year old pregnant female	Pica for p-DCB toilet blocks first trimester	General tiredness, mild anorexia, dizziness, edema of ankles	Hemolytic anemia	Healthy child delivered several months later	Campbell and Davidson, 1970

continuously exposed to fumes of cleaning solution containing 95% o-DCB and 5% p-DCB. She exhibited pallor, weakness, headaches, vomiting and severe pains. She was diagnosed as having a severe hemolytic anemia (1,500,000 RBCs/mm^3), accompanied by leukocytosis and polynucleosis, and the presence of some immature elements belonging to the granulocytic and erythrocytic series. Vigorous treatment and a change of employment resulted in essentially a full recovery.

Girard, et al. (1969) reported three cases of leukemia which they attributed to chronic exposure to o-dichlorobenzene (o-DCB). One man hospitalized for chronic lymphoid leukemia worked with a solvent containing 80% o-DCB and 15% p-DCB for 10 years. A girl hospitalized with acute myeloblastic leukemia died 10 months later of peripheral leukoblastosis. She reportedly had a neurotic compulsion to remove dirt and grease stains from her clothes, which she did repeatedly with a product containing 37% o-DCB (no benzene or toluene). Another man exposed to a glue containing 2% o-dichlorobenzene, methyl ethyl ketone and cyclohexane for a period of 29 years died of chronic lymphoid leukemia. No further details of these incidents were given.

Girard, et al. (1969) also reported the case of a 60-year old male, who for 30 years had worked in a job during which he had been in contact with mono-, o-di- and trichlorobenzene. At the time he was seen by the authors, he exhibited symptoms of weakness and tiredness. Clinical studies revealed that he suffered from anemia, with an erythrocyte count of 3 million/mm^3. No follow-up of this individual was described.

p-Dichlorobenzene

In cases where moderate exposures to p-dichlorobenzene were documented, patients complained of severe headaches, profuse rhinitis and periorbital swelling for approximately 24 hours after exposure (Cotter, 1953; Campbell and Davidson, 1970). Anorexia, nausea, vomiting, weight loss and yellow atrophy of the liver were reported for high exposure concentrations (Petit and Champeix, 1948; Cotter, 1953; Hallowell, 1959).

Wallgren (1953) reported loss of weight, exhaustion, decrease of appetite and blood dyscrasias in 27 men who manufactured p-DCB for 1 to 7 months. Cotter (1953) described the case of a woman who demonstrated products containing p-DCB and who complained of tiredness, nausea, vomiting and headache. Clinical studies showed that she had acute yellow atrophy and cirrhosis of the liver.

Heavy use of p-DCB as either a moth repellent or a deodorizer apparently resulted in weakness, nausea, vomiting of blood and jaundice (Perrin, 1941;Cotter,1953;Weller and Crellin,1953). One man and his wife died within months of each other of acute yellow atrophy of the liver (confirmed by autopsy) (Cotter, 1953). Their home apparently was saturated with p-DCB moth ball vapor for a period of at least three to four months.

There were at least two reports of deliberate ingestion of p-dichlorobenzene. One woman who developed a pica for p-DCB during the first trimester of her pregnancy complained of general fatigue, mild anorexia, dizziness and edema of the ankles. (Campbell and Davidson, 1970). She was hospitalized with hemolytic anemia, but delivered a healthy child several months later. Another woman who ingested 4-5 p-DCB pellets (size not indicated) daily for 2 1/2 years complained about increasing patchy pigmentation (Frank and Cohen, 1961). Unsteadiness and tremors occurred when she stopped taking the pellets; these symptoms were thought to be due to psychological rather than physiological withdrawal.

VII. MECHANISM(S) OF TOXICITY

Of the various toxic effects occurring after exposures to the dichlorobenzene, information on the mechanisms of toxicity is available only for 1) hepatotoxicity, which perhaps also can be applied to similar changes in the kidney and lung, and 2) for the induction of porphyria via acceleration of synthesis in the heme pathway.

Many workers have studied the possibility that cellular damage caused by many drugs and other xenobiotics is mediated via chemically-reactive metabolites. Many of the metabolites formed are chemically inactive, but certain of the metabolites such as the arene oxides or epoxides may interact with physiological or biochemical processes, causing either pharmacological or toxicological effects.

Studies of halogenated benzenes, including the dichlorobenzenes, indicate that hepatic necrosis produced on exposure to these compounds results from their conversion to reactive toxic intermediates. Reid and co-workers (1971,1973) have shown that an increase in toxicity can be correlated with an increase in covalent binding of metabolites to proteins within liver cells. This relationship can be seen both in vivo and in vitro. In the presence of the resulting hepatic necrosis, a decrease in available glutathione levels was observed as well as an increase in mercapturic acid excretion (see Table VII-1). This suggests that with sufficient depletion of glutathione (greater than 20-25% or higher), the liver loses its ability to "detoxify" the chemical by complexing with the substance to form a less reactive substance, and thus, proportionately more reactive metabolite is available to interact with tissue proteins

Table VII-1

Covalent Binding, Hepatotoxicity and Mercapturic Acid Excretion of Halogenated Benzene Derivatives

(Reid et al., 1971; Reid et al., 1973; Reid and Krishna, 1973)
(After Ware and West, 1977)

Compound		Covalent binding (nmols/mg protein ± S.E.) (N=6)	Hepatic necrosis	Mercapturic acid excretion	Glutathione concentration (% of control)*
Monobromobenzene	(1 mM/kg)	0.534 ± 0.050+	Yes	3 +	67
Monochlorobenzene	(1 mM/kg)	0.604 ± 0.044+	Yes	3 +	Not determined
Monoiodobenzene	(1 mM/kg)	0.323 ± 0.054	Yes	3 +	66†
Monofluorobenzene	(1 mM/kg)	0.060 ± 0.004+	No	+	82
o-Dichlorobenzene	(0.5 mM/kg)	0.234 ± 0.015§	Yes	2 +	48†
p-Dichlorobenzene	(0.5 mM/kg)	0.021 ± 0.002§	No	±	101

* Glutathione concentration determined 3 hours after administration of the hydrocarbon.

+ Killed at 24 hours

† P<0.01 compared with control.

§ Killed at 6 hours.

and other essential macromolecules, resulting in cellular and tissue damage. Stimulation of metabolism by pretreatment with phenobarbital potentiates covalent binding in rats, as can be seen in Table VII-2. Conversely, blocking metabolism by SKF-525A (2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride) or piperonyl butoxide (a pesticide synergist) prevents their hepatoxicity (Reid et al., 1973; Reid and Krishna, 1973).

Just as there are species difference in metabolism there are also differences in the rate and degree of metabolism of a halogenated benzene in different organs, i.e., lung may differ from kidney which differs from liver, etc. Phenobarbital (PB) does not increase covalent binding in the lung as shown for the liver if administered before exposure to o-DCB (Reid, et al., 1973; see Table VII-3). This is due to the fact that PB is a poor inducer in the lung. Nevertheless, both the lung and kidney exhibit pathology following exposure to the dichlorobenzenes (Hollingsworth, et al., 1956; Battelle-Columbus, 1979b).

The halobenzenes, the dichlorobenzenes among them, also have been shown to induce porphyria (Rimington and Ziegler, 1963; Carlson, 1977, Battelle-Columbus, 1978c, 1978i, 1979a, 1979b). This condition, a disturbance in porphyrin metabolism, is characterized by increased formation and excretion of porphyrin precursors, cutaneous photosensitivity, frequent hemolytic anemia and splenomegaly. Acute abdominal and nervous system manifestations may also occur. As was described in some detail earlier, exposure to the dichlorobenzenes leads to the induction of the mitochondrial enzyme, Δ -aminolevulinic acid synthetase, resulting in an increased

Table VII-2

Effect of Phenobarbital and SKF-525A Administration on Covalent Binding of Halogenated Benzene Derivatives to Rat Liver Protein *In Vivo* 6 Hours After Administration

(Reid *et al.*, 1973; Reid and Krishna, 1973)
(After Ware and West, 1977)

	Control (nM/mg protein ± S.E.) (N=6)	Phenobarbital (nM/mg protein ± S.E.) (N=6)	Phenobarbital + SKF-525A[a] (nM/mg protein ± S.E.) (N=6)
Monobromobenzene-^{14}C (1 mM/kg)	0.267 ± 0.034	0.550 ± 0.031[+]	0.136 ± 0.024[†]
Monochlorobenzene-^{14}C (1 mM/kg)	0.364 ± 0.053	1.268 ± 0.278[+]	0.438 ± 0.144[§]
Monoiodobenzene-^{14}C (1 mM/kg)	0.090 ± 0.015	0.545 ± 0.129[+]	0.666 ± 0.304
Monofluorobenzene-^{14}C (1 mM/kg)	0.085 ± 0.015	0.054 ± 0.008	0.052 ± 0.005
o-Dichlorobenzene-^{14}C (0.5 mM/kg)	0.234 ± 0.015	0.308 ± 0.038	0.186 ± 0.014[§]
p-Dichlorobenzene-^{14}C (0.5 mM/kg)	0.021 ± 0.002	0.012 ± 0.001[+]	0.006 ± 0.001[†]

[a] Diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF-525A) (75 mg/kg i.p.) was given 1 hour before the hepatotoxin.

[+] $P<0.01$ compared with controls.
[†] $P<0.01$ compared with phenobarbital alone.
[§] $P<0.02$ compared with phenobarbital alone.

Table VII-3

Binding of Aromatic Hydrocarbons in Rat Lung: Effect of Phenobarbital*

(Reid et al., 1973)
(Modified from Ware and West, 1977)

Compound	Time of Sacrifice (hour)	Binding of Hydrocarbon in Lung (mumole/mg protein)	
		Control	Phenobarbital
o-Dichlorobenzene-^{14}C,	6	27.3 ± 1.4	18.9 ± 2.7 ($p<0.05$)
0.5 mM/kg	24	20.9 ± 2.4	15.3 ± 2.2
p-Dichlorobenzene-^{14}C,	6	4.6 ± 0.2	3.4 ± 0.5
0.5 mM/kg	24	3.4 ± 0.4	1.8 ± 0.2

* Values are the means ± SE of 6 animals.

production from heme of aminolevulinic acid and then other constituents of the heme synthetic pathway (Rimington and Ziegler, 1963; Poland, et al., 1971; Ariyoshi, et al., 1975). When these levels exceed those needed for maintenance of the system, clinical symptoms may be manifested.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Adjusted Acceptable Daily Intake (AADI) for the chemical is determined. For ingestion data, this approach is illustrated as follows:

$$\text{Adjusted ADI} = \frac{(\text{NOAEL or MEL in mg/kg})(70\ \text{kg})}{(\text{Uncertainty factor})(2\ \text{liters/day})}$$

The 70 kg adult consuming 2 liters of water per day is used as the basis for the calculations. A "no-observed-adverse-effect-level" or a "minimal-effect-level" is determined from animal toxicity data or human effects data. This level is divided by an uncertainty factor because, for these numbers which are derived from animal studies, there is no universally acceptable quantitative method to extrapolate from animals to humans, and the possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human toxicity data, an uncertainty factor is used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxins. The guidelines set forth by the National Academy of Sciences (Drinking Water and Health, Vol. 1, 1977) are used in establishing uncertainty factors. These guidelines are as follows: an uncertainty factor of 10 is used if there exist valid experimental results on ingestion by humans, an uncertainty factor of 100 is used if there exist valid results on long-term feeding studies on experimental animals, and an uncertainty factor of 1000 is used if only limited data are available.

In the quantification of carcinogenic effects, mathematical models are used to calculate the estimated excess cancer risks associated with the consumption of a chemical through the drinking water. EPA's Carcinogen Assessment Group has used the multistage model, which is linear at low doses and does not exhibit a threshold, to extrapolate from high dose animal studies to low doses of the chemical expected in the environment. This model estimates the upper bound (95% confidence limit) of the incremental excess cancer rate that would be projected at a specific exposure level for a 70 kg adult, consuming 2 liters of water per day, over a 70 year lifespan. Excess cancer risk rates also can be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. Current understanding of the biological mechanisms involved in cancer do not allow for choosing among the models. The estimates of incremental risks associated with exposure to low doses of potential carcinogens can differ by several orders of magnitude when these models are applied. The linear, non-threshold multi-stage model often gives one of the highest risk estimates per dose and thus would usually be the one most consistent with a regulatory philosophy which would avoid underestimating potential risk.

The scientific data base, which is used to support the estimating of risk rate levels as well as other scientific endeavors, has an inherent uncertainty. In addition, in many areas, there exists only limited knowledge concerning the health effects of contaminants at levels found in drinking water. Thus, the dose-response data gathered at high levels of exposure are used for extrapolation to estimate responses at levels of exposure nearer to the range in which a standard might be set. In most cases, data exist only for animals; thus, uncertainty exists when the data are extrapolated to humans. When estimating risk rate levels, several other areas of uncertainty exist such as the effect of age, sex, species and target organ of the test animals used in the experiment, as well as the exposure mode and dosing rates. Additional uncertainty exists when there is exposure to more than one contaminant due to the lack of information about possible additive, synergistic or antagonistic interactions.

Non-Carcinogenic Effects

Among the toxic effects observed following exposure to the dichlorobenzenes in humans and other animals have been: central nervous system (CNS) depression, liver and kidney damage and blood dyscrasias (granulocytopenia, hemolytic anemia and leukemias). It remains unclear as to whether these latter effects are due to the DCBs themselves or a contaminant. In addition to liver necrosis, the dichlorobenzenes also can produce porphyria. The appearance and intensity of these and other adverse effects are dependent upon dose and duration of exposure. Death following high level acute exposure usually results from the CNS effects (primarily, respiratory failure).

Deaths in humans have been reported following accidental exposure.

Several investigators have determined the acute lethal dose levels after exposure to ortho- and para-dichlorobenzene in several species. These data are summarized in Table VIII-1.

A number of studies with o-dichlorobenzene and p-dichlorobenzene are available in which dose-response data are described and which allow the identification of no-observed-adverse-effect-levels (NOAELs). Animals were exposed both by gavage and by inhalation for periods of time constituting a subacute, subchronic or chronic exposure. Since several gavage studies are available for evaluation, only these, and not the inhalation studies, will be used in the quantification of toxicological effects. The oral route of exposure is more appropriate for the development of allowable exposure levels in drinking water. Comparable studies with the meta- isomer of dichlorobenzene have not been reported. Therefore, it will be assumed that ADIs developed for o-dichlorobenzene also will be appropriate for m-dichlorobenzene. This assumption is defensible for two reasons: 1) in general, in mutagenicity and other short-term tests, the meta isomer behaved more like the ortho isomer than like the para isomer, and 2) short- and longer-term studies with o- and p-DCB suggest that the ortho isomer is somewhat more toxic than the para isomer on a mg/kg basis. Thus, to assume that the meta isomer is more similar to the ortho isomer would be consistent with a regulatory philosophy that seeks to avoid underestimating the potential risk to human health.

Table VIII-1

Acute Toxicity Data for o- and p-Dichlorobenzene

Animal	Route	LD_{50}	LC_{Lo}	Reference
o-Dichlorobenzene				
Rat	Oral	500 mg/kg		NIOSH, 1978
Guinea Pig	Oral	2000 mg/kg		Hollingsworth, *et al*., 1958
Rat	Inhal.		821 ppm/7 hr	Hollingsworth, 1958
Guinea Pig	Inhal.		800 ppm/7 hr	Hollingsworth, 1958
Guinea Pig	Inhal.		800 ppm/24 hr	Cameron, *et al*., 1937
p-Dichlorobenzene				
Rat	Oral	500 mg/kg		NIOSH, 1978
Rat	Oral	2500 mg/kg		Hollingsworth, *et al*., 1956
Guinea Pig	Oral	2800 mg/kg (LD_{Lo})		Hollingsworth, *et al*., 1956
Mouse	SC	5145 mg/kg		Irie, *et al*., 1973

o-Dichlorobenzene (and/or m-Dichlorobenzene)

1. One-day Health Advisory (1-day HA)

No satisfactory dose-response data are available from which to derive a 1-day HA for either the 10 kg child or the 70 kg adult. It is recommended that for this duration of exposure the 10-day HA be applied (see below).

2. Ten-day Health Advisory (10-day HA)

Three studies are available from which NOAELs can be identified and which might be appropriate for use in the development of the 10-day HA.

A 14-day study with o-DCB was conducted in Fischer 344 rats are part of the prechronic test phase of the NTP bioassay (Battelle Columbus, 1978d). Oral gavage doses in corn oil of 60, 125, 250, 500 or 1000 mg/kg were administered to five animals/sex/group. Controls received corn oil alone. The parameters measured were death, body weight gain and gross examination of all rats and microscopic examination in only some. A NOAEL of 125 mg/kg was identified, based upon decreased body weight gain in animals dosed at 250 and 500 mg/kg.

If these data were used to develop a 10-day HA, the HA would be derived as follows:

For the 10 kg child:

$$\frac{125 \text{ mg/kg/day} \times 10 \text{ kg} \times 1.0}{100 \times 1 \text{ l}} = 12.5 \text{ mg/l}$$

Where: 125 mg/kg = NOAEL, based upon lack of significant effect on body weight gain in rats

10 kg = weight of protected individual

1.0 = ratio of dose absorbed

100 = uncertainty factor, appropriate for use with NOAEL from animal data and no comparable human data

1 l = 1 liter, assumed volume of water consumed per day by 10 kg child

For the 70 kg adult:

$$\frac{125\ \text{mg/kg} \times 70\ \text{kg} \times 1.0}{100 \times 2\ \text{l}} = 43.75\ \text{mg/l}$$

Where: 70 kg = weight of protected individual

2 l = 2 liters, assumed volume of water consumed per day by 70 kg adult

(Other factors as described above for 10 kg child)

Subchronic gavage studies with o-DCB in mice and rats were conducted to assist in dosage selection for the NTP 104-week carcinogenicity study (Battelle-Columbus, 1978c,i). Single doses in corn oil were administered 5 days/week for 13 weeks. Treated animals received doses of 30, 60, 125, 250 or 500 mg/kg/day. Controls received corn oil. Other protocol details are described in Chapter V.

In the mice, body weight gain was decreased significantly in animals of both sexes at the 500 mg/kg dose level and in males at the 250 mg/kg dose. Of the hematological parameters tested, white cell counts of treated males were higher than those of control males. It was suggested that this was due to lower than normal control values, as observed typically in that laboratory. Since individual data were not available for statistical analysis, it cannot be determined whether or not the differences between the controls and the treated groups were statistically significant. But, since there is at least anecdotal evidence to suggest a possible relationship between exposure to o-DCB and leukemia in humans, it would be prudent to evaluate these results in greater depth.

Increased, but apparently not statistically-significant, blood alkaline phosphatase levels were observed in male mice receiving 125 and 250 mg o-DCB/kg/day. SGPT levels were increased significantly in the two surviving males receiving 500 mg/kg/day, due to the high value for the one animal exhibiting hepatocellular necrosis. Urine volume was greater in the treated animals than in controls, but no record of fluid intake was kept. The significance of this observation is unknown, since no urinalysis parameters were altered, except for the decreases in specific gravity and creatinine levels.

Male mice receiving 500 mg o-DCB/kg exhibited higher uroporphyrin levels than did male controls; females at that dose showed higher coproporphyrin levels. These parameters were not measured in the other dose groups. In addition, a dose-dependent increase in liver protoporphyrin was observed in the females, but not in the males. Even without statistical evaluation, one could observe that liver weights in the highest dose group of both sexes were increased significantly. An increase of lesser magnitude was observed in the females at the 250 mg/kg/day dose level. Histopathological examination of several tissues revealed that no lesions were apparent in animals treated with 125 mg/kg/day or lower doses of o-DCB.

Interpretation of the results of the study suggests that an oral NOAEL of 125 mg/kg/day could be identified in mice, if one discounts the observations concerning the white cell counts. In the absence of the raw data, at this time, it will be assumed that the investigators have interpreted this finding correctly.

If one were to assume that the results of the mouse 90-day study were appropriate for use in developing a 10-day HA, it could be derived thusly:

For the 10 kg child:

$$\frac{125\ \text{mg/kg/day} \times 10\ \text{kg} \times 1.0}{100 \times 1\ \text{l}} \times \frac{5}{7} = 8.93\ \text{mg/l}$$

Where: 125 mg/kg/day = NOAEL, based upon lack of histological evidence of toxicity in mice

10 kg = weight of protected individual

1.0 = ratio of dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week exposure pattern

100 = uncertainty factor, appropriate for use with NOAEL from animal data and no comparable human data

1 l = 1 liter, assumed volume of water consumed per day by 10 kg child

For the 70 kg adult:

$$\frac{125\ \text{mg/kg/day} \times 70\ \text{kg} \times 1.0 \times 5}{100 \times 2\ \text{l} \times 7} = 31.25\ \text{mg/l}$$

Where: 70 kg = weight of protected individual

2 l = 2 liters, assumed volume of water consumed per day by 70 kg adult

(Other factors as described above for 10 kg child)

In the rats, body weight gain was decreased significantly at the two highest doses (250 and 500 mg/kg/day). Cholesterol levels were increased in males at the two highest doses and in females at the three highest doses. The combined alpha-globulin fraction appeared to be increased in females at the highest dose and males at the two highest doses. As in the mice, urinary output was increased substantially, with concomitant decreases in specific gravity and creatinine.

Both uro- and coproporphyrin levels in the urine increased significantly in animals receiving 500 mg/kg/day. No measurements of these parameters were made in other dose groups. However, liver protoporphyrin levels were not changed. Absolute and relative liver weights were increased in the 250 and 500 mg/kg/day groups. Histopathological examination of tissues revealed liver and kidney changes in the highest dose group, and liver changes in the 250 mg/kg/day groups. As for the mice, 125 mg/kg/day was identified as the NOAEL for the rats.

If one were to assume that the results of the study in which rats were exposed to o-DCB subchronically were appropriate for use in developing a 10-day HA, it could be derived thusly:

For the 10 kg child:

$$\frac{125 \text{ mg/kg/day} \times 10 \text{ kg} \times 1.0 \times 5}{100 \times 1 \text{ l} \times 7} = 8.93 \text{ mg/l}$$

Where: 125 mg/kg/day = NOAEL in rats, based upon lack of histological evidence of liver damage

10 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week exposure

100 = uncertainty factor, appropriate for use with NOAEL from animal data and no comparable human data

1 l = 1 liter, assumed volume of water consumed per day by 10 kg child

For 70 kg adult:

$$\frac{125 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 2 \text{ l} \times 7} = 31.25 \text{ mg/l}$$

Where: 70 kg = weight of protected individual

2 l = 2 liters, assumed volume of water consumed per day by 70 kg adult

(Other factors as described above for 10 kg child)

3. ADI (Acceptable Daily Intake)

Several studies are available in which NOAELS have been identified from dose-response data in experimental animals and which are of sufficient duration to be considered candidates from which an ADI for non-carcinogenic effects could be derived for o-DCB (and, m-DCB).

Varshavskaya (1968) administered o-DCB orally to white rats for nine months at doses of 0.001, 0.01 or 0.1 mg/kg/day. Effects were observed at the two higher doses; none were observed at the 0.001 mg/kg/day dose. While this study suggests that effects can be seen at very low doses when compared with the other studies, little in the way of quantitative experimental detail was presented in her publication. Therefore, it is difficult to assess fully the results presented, and one cannot conclude that this paper should be the basis for the development of an ADI for o-DCB (and, m-DCB).

Hollingsworth, et al. (1958) gave rats a series of 138 doses of o-DCB over a period of 192 days (18.8, 188 or 376 mg/kg/day, five days a week) by intubation. No adverse effects were observed at the lowest dose. With the intermediate dose, a slight increase in liver and kidney weight was noted. At the highest dose, there was a slight decrease in the weight of the spleen and a modest increase in the weight of the liver accompanied by cloudy swelling.

If one were to assume that the results of this study were appropriate for use in developing an acceptable daily intake (ADI), it would be derived thusly:

$$\frac{18.8\ \text{mg/kg/day} \times 70\ \text{kg} \times 1.0 \times \frac{5}{7}}{100 \times 10} = 0.94\ \text{mg/day}$$

Where: 18.8 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Subchronic gavage studies with o-DCB in mice and rats were conducted to assist in dosage selection for the NTP 104-week carcinogenicity study (Battelle-Columbus, 1978c,i). Single doses in corn oil were administered 5 days/week for 13 weeks. Treated animals received doses of 30, 60, 125, 250 or 500 mg/kg/day. Controls received corn oil. Other protocol details are described in Chapter V.

The results of the study in mice are detailed in the section on the development of the 10-day Health Advisories. Interpretation of these results suggests an oral NOAEL of 125 mg/kg/day could be identified in mice. If one were to assume that this NOAEL was appropriate for use in developing an ADI for o-DCB, the ADI could be derived thusly:

$$\frac{125\ \text{mg/kg/day} \times 70\ \text{kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 6.25\ \text{mg/day}$$

Where: 125 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration of significantly less than lifetime

The results of the subchronic study in rats also are detailed in the section on the development of the 10-day Health Advisories. As for the mice, 125 mg/kg/day was identified as the NOAEL. If one were to assume that this NOAEL were appropriate for use in developing an ADI for o-DCB (and, m-DCB), the ADI could be derived thusly:

$$\frac{125 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 6.25 \text{ mg/day}$$

Where: 125 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Chronic (104-week) gavage studies with o-DCB in mice and rats were conducted in the NTP carcinogenicity study (NTP, 1982). The results were presented in a preliminary report not yet approved by the NTP Board of Scientific Counselors. Single daily doses of o-DCB in corn oil were administered 5 days/week. Treated animals of both species received doses of 60 or 120 mg/kg/day. Controls received corn oil alone. Other protocol details are described in Chapter V.

In the mice, no differences in survival rates were observed between treated and control groups of either sex. There also was no evidence of any compound-related non-neoplastic liver lesions, suggesting that the maximum tolerated dose was not administered in this study and that a higher dose could have been tolerated.

If the results of the mouse chronic study were considered appropriate for use in developing an ADI, it could be derived thusly:

$$\frac{120 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 7} = 60 \text{ mg/day}$$

(for a 70 kg adult)

Where: 120 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (lifetime exposure)

In the rats during the NTP bioassay, there was no significant difference in the survival rates of female rats at either dose or of males at the low dose when compared with controls. However, there was a statistically significant decrease in survival in the high dose males($P < 0.001$). This difference appeared to be due, primarily, to gavage error. As with the mice, there were no non-neoplastic lesions of the liver in rats of either sex receiving either dose of o-DCB. Again, this suggests that the maximum tolerated dose was not achieved and a higher dose should have been administered.

If the results of the rat chronic study were considered appropriate for use in developing an ADI, it could be derived thusly:

$$\frac{120 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 7} = 60 \text{ mg/day}$$

(for a 70 kg adult)

Where: 120 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (lifetime exposure)

p-Dichlorobenzene

1. One-day Health Advisory (1-day HA)

No satisfactory dose-response data are available from which to derive a 1-day HA for p-DCB for either the 10 kg child or the 70 kg adult. It is recommended that for this duration of exposure, the 10-day HA be applied (see below).

2. Ten-day Health Advisory (10-day HA)

Several studies are available from which NOAELS can be identified and which might be appropriate for use in the development of the 10-day HAs.

A 14-day study with p-DCB was conducted in Fischer 344 rats as part of the prechronic test phase of the NTP bioassay for that compound (Battelle Columbus, 1978f). Gavage doses of 60, 125, 250, 500 or 1000 mg p-DCB/kg in corn oil were administered to five males and five females per group. Controls were untreated. The parameters measured were death, body weight gain and gross examination of organs at necropsy. A NOAEL of 250 mg/kg was identified, based upon the lack of significant effect upon body weight gain.

If these data were used to develop a 10-day HA, the HA would be derived as follows:

For the 10 kg child:

$$\frac{250\ \text{mg/kg/day} \times 10\ \text{kg} \times 1.0}{100 \times 1\ \text{l}} = 25\ \text{mg/l}$$

Where: 250 mg/kg/day = NOAEL, based upon lack of signi- effect upon body weight gain in rats

10 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

100 = uncertainty factor, appropriate for use with NOAEL from animal data and no comparable human data

1 l = 1 liter, assumed volume of water consumed per day by 10 kg child

For the 70 kg adult:

$$\frac{250 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0}{100 \times 2 \text{ l}} = 87.5 \text{ mg/l}$$

(may exceed solubility in water)

Where: 70 kg = weight of protected individual

2 l = 2 liters, assumed volume of water consumed per day by 70 kg adult

(Other factors as described above for 10 kg child)

Subchronic gavage studies with p-DCB in mice and rats were conducted to assist in dosage selection for the NTP 104-week carcinogenicity study (Battelle-Columbus, 1979a,b;1980a,b). The 1979 studies had to be repeated because a no-effect level could not be identified in mice receiving 600, 900, 1000, 1500 or 1800 mg/kg/day or in rats receiving 300, 600, 900, 1200 or 1500 mg/kg/day. In the repeat subchronic studies, the animals received single doses of p-DCB in corn oil 5 days/week for 13 weeks. Treated mice were administered doses of 84.4, 168.8, 337.5, 675 or 900 mg p-DCB/kg/day; rats were administered 37.5, 75, 150, 300 or 600 mg p-DCB/kg/day. Controls received corn oil. Other protocol details are described in Chapter V.

In the mice, no significant differences were apparent in dietary consumption or relative body weight gains between treated and control animals of either sex. Upon histopathological examination, it was observed that animals in the two highest dose groups exhibited a significant degree of centrolobular to midzonal hepatocytomegaly. Few changes of this nature were observed in animals receiving 337.5 mg/kg/day. This dose was determined to be the NOAEL.

If the results of this study in mice were considered appropriate for use in developing a 10-day HA, it would be derived thusly:

For the 10 kg child:

$$\frac{337.5\ \text{mg/kg/day} \times 10\ \text{kg} \times 1.0 \times 5}{100 \times 1\ \text{l} \quad \times \quad 7} = 24.1\ \text{mg/l}$$

Where: 337.5 mg/kg/day = NOAEL, based upon no significant histopathology

10 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week exposure

100 = uncertainty factor, appropriate for use with NOAEL from animal data and no comparable human data

1 l = 1 liter, assumed volume of water consumed by 10 kg child

For the 70 kg adult:

$$\frac{337.5\ \text{mg/kg/day} \times 70\ \text{kg} \times 1.0 \times 5}{100 \times 2\ \text{l} \times 7} = 84.4\ \text{mg/l}$$

Where: 70 kg = weight of protected individual

2 l = 2 liters, assumed volume of water consumed per day by 70 kg adult

(Other factors as described above for 10 kg child)

In the rats, no significant differences in food consumption or body weight gain were observed between treated and control animals of either sex at any dose. Microscopically, there was an increased incidence and severity of renal cortical degeneration in males receiving the two highest doses. Females showed no significant changes at any dose level. The NOAEL for this study was established at 150 mg/kg/day.

If the results of this rat subchronic study were considered for use in developing the 10-day Health Advisories, the HAs would be derived thusly:

For the 10 kg child:

$$\frac{150\ \text{mg/kg/day} \times 10\ \text{kg} \times 1.0 \times 5}{100 \times 1\ \text{l} \times 7} = 10.7\ \text{mg/l}$$

Where: 150 mg/kg/day = NOAEL, based of lack of significant histopathology

10 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week exposure

100 = uncertainty factor, appropriate for use with NOAEL from animal data and no comparable human data

1 l = 1 liter, assumed volume of water consumed per day by 10 kg child

For the 70 kg adult:

$$\frac{150 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 2 \text{ l} \times 7} = 37.5 \text{ mg/l}$$

Where: 70 kg = weight of protected individual

2 l = 2 liters, assumed volume of water consumed per day by 70 kg adult

(Other factors as described above for 10 kg child)

3. ADI (Acceptable Daily Intake)

Several studies are available in which NOAELs have been identified from dose-response data in experimental animals and which are of sufficient duration to be considered candidates from which an ADI for non-carcinogenic effects could be derived for p-DCB.

Hollingsworth, _et al_. (1956) administered 138 oral doses of p-DCB at 18.8, 188 or 376 mg/kg/day to rats over a 192-day period (5 days/week). At the highest dose (376 mg/kg/day), there was an increase in the weights of the spleen, kidney and liver accompanied by slight cirrhosis and focal necrosis in the liver. There was also cloudy swelling of the renal tubular epithelium. At the intermediate dose (188 mg/kg/day), liver and kidney weights were increased. No adverse effects were noted at the lowest dose (18.8 mg/kg/day).

If one were to assume that the results of this study were appropriate for use in developing an acceptable daily intake (ADI), it would be derived thusly:

$$\frac{18.8 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 94 \text{ mg/day}$$

Where: 18.8 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Subchronic studies in mice and rats were conducted with p-DCB administered by gavage in the prechronic phase of the NTP bioassay (Battelle Columbus, 1979a,b,;1980a,b). Groups of ten animals/sex/dose were given p-DCB in corn oil 5 days/week for 13 weeks. Control animals received corn oil alone. Mice were dosed at levels of 84.4, 168.8, 337.5, 675 or 900 mg/kg/day. Rats received 37.5, 75, 250, 300 or 600 mg/kg/day.

The results of the study in the mice are detailed in the section on the development of the 10-day Health Advisories. The NOAEL in this study was identified as 337.5 mg/kg/day.

If one were to assume that this NOAEL were appropriate for use in developing an ADI for p-DCB, then the ADI would be derived thusly:

$$\frac{337.5\ \text{mg/kg/day} \times 70\ \text{kg} \times 1.0 \times 5}{100 \times 10 \times \qquad 7} = 16.9\ \text{mg/day}$$

Where: 337.5 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

The results of the study in rats also are described in detail in the section on the development of the 10-day Health Advisories. The NOAEL determined from this study was 150 mg/kg/day. If this NOAEL were used to derive an ADI for p-DCB, the ADI would be developed as follows:

$$\frac{150\ \text{mg/kg/day} \times 70\ \text{kg} \times 1.0 \times 5}{100 \times 10 \qquad 7} = 7.5\ \text{mg/day}$$

Where: 150 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Existing Guidelines and Standards

[to be added]

Quantification of Non-carcinogenic Effects

Table VIII-2 summarizes the 10-day HAs derived from the three studies on o- and p-dichlorobenzene which contain adequate dose-response data for this duration of exposure. Even though the 14-day studies were conducted over a time course roughly identical to the HA duration, they are not the best choice for derivation of the 10-day HAs. This is because the parameters measured (death, body weight gain and gross examination) are less sensitive indicators of toxicity than are the histopathology and special parameters included in the subchronic studies. Therefore, while the 14-day data are supportive to a certain degree, in this case, the subchronic studies provide a more accurate picture of the toxic potential of these compounds over the 10-day period. In addition, as can be noted, the rat appears to be more sensitive to the effects of p-dichlorobenzene. Thus, it is recommended that the 10-day HA derived from the rat subchronic study be used as guidance in the event of contamination by this isomer.

TABLE VIII-2

Possible Health Advisories for o-, m- and p-Dichlorobenzene

Compound/Study	10 kg child	70 kg adult
	(mg/l)	
o-Dichlorobenzene (and, meta-)		
14-day subacute	12.5	43.8
90-day subchronic (mouse)	8.9	31.2
90-day subchronic (rat)	8.9	31.2
p-Dichlorobenzene		
14-day subacute	25	87.5
90-day subchronic (mouse)	24.1	84.4
90-day subchronic (rat)	10.7	37.5

Table VIII-3 summarizes the ADIs derived from the available gavage studies on o- and p-dichlorobenzene which contain adequate dose-response data identifying NOAELs. As can be seen, a wide range of numbers was derived. For o-DCB (and m-DCB), the ADIs range from 0.94 mg/day (Hollingsworth, et al., 1958) to 60 mg/day (NTP, 1982;preliminary report). For p-DCB, the ADIs range from 0.94 mg/day (Hollingsworth, et al., 1956) to 16.9 mg/day (Battelle-Columbus, 1980a). However, a rationale can be presented by which certain of these numbers can be eliminated and others supported.

Ortho-dichlorobenzene (and, meta-dichlorobenzene)

The fact that the ADIs generated from the chronic studies in the NTP bioassay are larger than those derived from the subchronic studies preceding them would suggest that the 10-fold uncertainty factor used to estimate a chronic ADI from subchronic data may be unnecessarily large for this compound. However, one reason that the chronic studies yielded larger ADIs than did the subchronic studies may be that the "special" parameters (i.e. urinalysis, clinical chemistry, hematology) were not included in the chronic study protocols. Had more sensitive indices of toxicity been evaluated in the chronic studies, effects may have been noted at one or the other of the doses which were utilized. In light of this consideration, it may not be prudent to suggest that the 10-fold uncertainty factor is too large. In addition, the NTP Board of Scientific Counselors has not yet approved the report prepared on the

TABLE VIII-3

Possible ADIs for the Dichlorobenzenes

Compound	Experiment	Possible ADI
o-DCB/m-DCB	Hollingsworth, et al. (1958) subchronic rat	0.94 mg/day
	Battelle-Columbus (1978c) subchronic mouse	6.25 mg/day
	Battelle-Columbus (1978i) subchronic rat	6.25 mg/day
	NTP (1982) chronic mouse (preliminary report)	60 mg/day
	NTP (1982) chronic rat (preliminary report)	60 mg/day
p-DCB	Hollingsworth, *et al*. (1956) subchronic rat	0.94 mg/day
	Battelle-Columbus (1980a) subchronic mouse	16.9 mg/day
	Battelle-Columbus (1980b) subchronic rat	7.5 mg/day

bioassay of o-DCB. Therefore, it is prudent to reserve judgment on its validity until such time as the report is approved.

The results of Hollingsworth, et al. (1958) suggest an ADI of 0.94 mg/day while those of the subchronic studies preceding the NTP bioassay suggest an ADI of 6.25 mg/day. Each ADI was derived from a NOAEL (18.8 mg/kg vs. 125 mg/kg, respectively). Since the highest NOAEL should be used to derive an ADI, it is more appropriate to use the NOAEL established in the NTP subchronic studies than the NOAEL from the Hollingsworth study. In addition, the NTP subchronic studies employed additional doses (5 vs 3), thereby allowing for a more precise identification of a NOAEL. It also should be noted that the minimal effect dose identified in the Hollingsworth study (188 mg/kg) is somewhat higher than the NOAEL established in the NTP subchronic studies.

For o-DCB (and, m-DCB), then, if one were to use the ADI from the NTP subchronic studies to determine the AADI, it would be derived thusly:

$$\text{AADI} = \frac{\text{ADI}}{2\ \text{l}} = \frac{6.25\ \text{mg/day}}{2\ \text{l}} = 3.125\ \text{mg/l/day}$$

This AADI assumes that the protected individual (a 70 kg adult) drinks 2 liters of water/day and that the sole source of exposure to o- or m-dichlorobenzene is via that drinking water. [It is important to note that the odor threshold for o-DCB and m-DCB in water has been identified as 0.01 and 0.02

ppm, respectively (Kolle, 1972). Therefore, any MCL for these compounds may have to consider the asthetic, as well as the toxic, consequences of exposure to these compounds in drinking water].

p-Dichlorobenzene

The results of the Hollingsworth, et al. (1956) study suggest an ADI of 0.94 mg/day while those from the subchronic studies preceding the NTP bioassay suggest ADIs of 7.5 mg/day (rats) and 16.9 mg/day (mice). It is apparent from these three studies, as well as the acute toxicity studies described earlier, that the rat is somewhat more sensitive to p-DCB toxicity than is the mouse. Therefore, to be consistent with the philosophy that one uses data from the most sensitive animal species when estimating the potential risk to the human, the data from the experiments in the rat should be used in deriving an AADI.

The ADI derived from the Hollingsworth study was based upon a NOAEL of 18.8 mg/kg; the ADI from the NTP subchronic study in the rat was derived from a NOAEL of 150 mg/kg. Since the highest NOAEL should be used to derive an ADI, it is more appropriate to use the NOAEL established in the NTP subchronic study than the NOAEL from the Hollingsworth study. The NTP subchronic study employed additional treatment doses (5 vs. 3), thereby allowing for a more precise identification of an NOAEL. In addition, it should be noted that the minimal effect level identified in the Hollingsworth study (188 mg/kg) was somewhat higher than the NOAEL established in the NTP subchronic study.

As with o-DCB (and m-DCB), it may be that any ADIs derived from the as yet unreported NTP chronic studies would be larger than those derived from the subchronic studies preceding them because the 10-fold uncertainty factor applied to accommodate for the difference in duration of exposure may be unnecessarily large. However, the same caveat should be considered as mentioned for o-DCB with reference to the lack of "special study" parameters being included in the chronic study. In addition, as with o-DCB until the report has been approved by the NTP Board of Scientific Counselors and published as final, it is appropriate to develop an ADI and AADI based upon the subchronic data. The AADI can be reevaluated at a later date.

For p-DCB, then, if one were to use the ADI from the NTP subchronic study in rats to determine the AADI, it would be derived thusly:

$$\text{AADI} = \frac{\text{ADI}}{2\ \text{l}} = \frac{7.5\ \text{mg/day}}{2\ \text{l}} = 3.75\ \text{mg/l/day}$$

This AADI assumes that the protected individual (a 70 kg adult) drinks 2 liters of water/day and that the sole source of exposure to p-dichlorobenzene is via that drinking water. [It is important to note that an odor threshold for p-DCB in water has been identified as 0.03 mg/l (Kolle, 1972). Therefore, any MCL for this compound may have to consider the asthetic, as well as the toxic, consequences of exposure to p-dichlorobenzene in drinking water].

Carcinogenic Effects

Both o-DCB and p-DCB have been administered by gavage in studies of their carcinogenic potential in F344 rats and B6C3F1 mice in the NTP Bioassay Program. A draft report of the results of the studies with o-DCB is available (NTP, 1982). A report of the results of the studies with p-DCB has not been made available as yet.

The preliminary assessment of the data from the studies on o-DCB suggests that, under the test conditions, o-DCB does not possess carcinogenic potential. However, until the NTP Board of Scientific Counselors approves the draft report, this assessment must remain preliminary.

Since no report, preliminary or otherwise, is available on the results of the studies with p-DCB, no assessment of its carcinogenic potential can be made at this time.

Quantification of Carcinogenic Effects

Preliminary assessment of the NTP Bioassay on o-DCB suggests that it was not carcinogenic under the conditions of the experiment.

No adequate data are available to assess the potential cancer risk following exposure to m- or p-DCB.

Summary

A summary of the Health Advisories and AADIs proposed for the three isomers of dichlorobenzene are shown in Table VIII-4.

TABLE VIII-4

Summary of Quantification of Toxicological Effects

Situation	Drinking Water Concentration (mg/l) 10 kg child	Drinking Water Concentration (mg/l) 70 kg adult	Reference
Ortho-dichlorobenzene (and, meta-)			
1-day HA	8.9	31.2	Battelle, 1978i
10-day HA	8.9	31.2	"
AADI		3.12	"
Excess Cancer Risk Estimate	(no indication of carcinogenic potential)		
Para-dichlorobenzene			
1-day HA	10.7	37.5	Battelle, 1980b
10-day HA	10.7	37.5	"
AADI		3.75	"
Excess Cancer Risk Estimate	(report not available;assessment not performed)		

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